Retrospective Genomic Characterization of a 2017 Dengue Virus Outbreak, Burkina Faso

Andrew G. Letizia,[1] Catherine B. Pratt,[1] Michael R. Wiley, Anne T. Fox, Mba Mosore, Bright Agbodzi, Clara Yeboah, Selassie Kumordjie, Nicholas Di Paola, Kone Cisse Assana, David Coulidiaty, Casimir Ouedraogo, Joseph H. Kofi Bonney, William Ampofo, Zékiba Tarnagda, Lassana Sangaré

Knowledge of contemporary genetic composition of dengue virus (DENV) in Africa is lacking. By using next-generation sequencing of samples from the 2017 DENV outbreak in Burkina Faso, we isolated 29 DENV genomes (5 serotype 1, 16 serotype 2 [DENV-2], and 8 serotype 3). Phylogenetic analysis demonstrated the endemic nature of DENV-2 in Burkina Faso. We noted discordant diagnostic results, probably related to genetic divergence between these genomes and the Trioplex PCR. Forward and reverse1 primers had a single mismatch when mapped to the DENV-2 genomes, probably explaining the insensitivity of the molecular test. Although we observed considerable homogeneity between the Dengvaxia and TetraVax-DV-TV003 vaccine strains as well as B cell epitopes compared with these genomes, we noted unique divergence. Continual surveillance of dengue virus in Africa is needed to clarify the ongoing novel evolutionary dynamics of circulating virus populations and support the development of effective diagnostic, therapeutic, and preventive countermeasures.

Dengue virus (DENV), the causative agent of dengue fever, is a mosquitoborne single-stranded RNA virus from the genus *Flavivirus*, often defined as 4 related serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) (*1*). Globally, ≈4 billion persons in 128 countries are at risk for dengue fever (*2*). An estimated 390 million infections occur annually, of which 96 million are symptomatic (*3*), making DENV the most prevalent and rapidly spreading mosquitoborne viral disease of human beings (*4*). Clinical manifestations vary from a self-limited, potentially debilitating illness to hypovolemic shock; the mortality rate can be as high as 20% if left untreated (*4*).

An estimated 750 million persons are at risk for acquiring DENV in Africa, and the disease burden is estimated to be nearly equivalent to that of the Americas (*3*,*5*). Many countries in Africa lack a national surveillance system and reporting mechanism (*6*), causing dengue fever cases to be misdiagnosed as malaria (*7*), which might explain why among the 34 countries in Africa to report dengue fever, 12 were not reported by the country where it occurred but by travelers returning to their country of origin (*8*). Travel, particularly to Africa, is emerging as a well-recognized mechanism of intercontinental DENV spread (*9*,*10*).

Less than 1% of all global DENV envelope sequence data, key information for vaccine targets, come from isolates from Africa (*11*). A need exists for additional DENV sequencing, especially in Africa (*12*,*13*). The lack of genomic DENV data from Africa combined with complex transmission dynamics involving urban and sylvatic cycles impairs our understanding of DENV's evolutionary history, transmission and spread (*13*), molecular diagnostics (*14*), antiviral targets (*15*), vector susceptibility (*16*), human immune response (*17*), vaccine development (*17*), and DENV spillover events (*18*). Determining which contemporary genotypes are in circulation is crucial to ensuring effective diagnostics and developing preventive and therapeutic countermeasures (*19*).

Author affiliations: Naval Medical Research Unit TWO, Singapore (A.G. Letizia); University of Nebraska Medical Center, Omaha, Nebraska, USA (C.B. Pratt, M.R. Wiley); Naval Medical Research Unit THREE, Ghana Detachment, Accra, Ghana (A.T. Fox); Noguchi Memorial Institute for Medical Research, Accra (M. Mosore, B. Agbodzi, C. Yeboah, S. Kumordjie, J.H.K. Bonney, W. Ampofo); US Army Medical Research Institute of Infectious Disease, Frederick, Maryland, USA (N. Di Paola); Institut de Recherche en Sciences de la Santé, Bobo-Dioulasso, Burkina Faso (K.C. Assana, Z. Tarnagda); Centre Hospitalier Universitaire Yalgado Ouédraogo, Ouagadougou, Burkina Faso (D. Coulidiaty, C. Ouedraogo, L. Sangaré)

DOI: https://doi.org/10.3201/eid2806.212491

[1]These first authors contributed equally to this article.

Burkina Faso, a country in West Africa with a population of ≈21 million persons, has had documented dengue fever outbreaks since 1925; known subsequent outbreaks occurred in 1982 and 2013 (*20*). In 2016, the World Health Organization declared an outbreak identifying 1,061 probable cases, primarily in the capital of Ouagadougou, population ≈2.5 million persons, in a setting of minimal surveillance and limited diagnostic ability (*21*). A larger outbreak, primarily in the central region that includes Ouagadougou, but involving all 13 health regions, occurred during August–November 2017, when Burkina Faso reported 9,029 suspected cases (*22*). Previous serotyping was conducted on 72 samples and demonstrated DENV-2 (58 cases), DENV-3 (12 cases), and DENV-1 (2 cases) (*23*); co-circulation of 3 serotypes occurred in Ouagadougou. The only published DENV genomes from either of these outbreaks were serotype 2, genotype Cosmopolitan, occurring after exposure during the 2016 outbreak among travelers returning to Japan and France (*24*,*25*).

By using in silico analyses, we determined whether unique DENV molecular divergence is occurring in Burkina Faso and assessed its impact on diagnostic assays and potential efficacy of vaccines and therapeutics. We sequenced DENV genomes from the 2017 outbreak in Burkina Faso to determine the molecular epidemiology of DENV and assess the homogeneity with targets for the US Centers for Disease Control and Prevention (CDC) Trioplex real-time reverse transcription PCR (RT-PCR), Dengvaxia (Sanofi Pasteur (https://www.sanofi.com) and TetraVax-DV-TV003 (Butantan Institute (http://butantan.gov.br) vaccine strains, and DENV antiviral epitopes.

Methods

Sample Processing and Sequencing

We obtained 791 deidentified human serum samples from patients with illness meeting the World Health Organization's clinical case definition of dengue fever during the 2017 DENV outbreak in Burkina Faso (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/28/6/21-2491-App1.pdf). Samples were provided by the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo-Dioulasso and Centre Hospitalier Universitaire Yalgado Ouédraogo in Ouagadougou. We processed the samples at Noguchi Memorial Institute of Medical Research in Accra, Ghana.

We tested each sample by using molecular and serologic techniques, and if any test consistent with acute infection was positive, we selected that sample for genome sequencing (Appendix Figure 1). We conducted molecular-based evaluation for DENV by using the CDC Trioplex assay after extraction with QIAamp viral RNA mini kits (QIAGEN, https://www.qiagen.com) according to the manufacturer's instructions. Serologic analyses included the detection of nonstructural protein 1 (NS1) antigen, DENV IgM, and DENV IgG (SD Bioline Dengue Duo; Abbott, https://www.globalpointofcare.abbott). We sequenced samples on an Illumina MiSeq (https://www.illumina.com) by using an enrichment-based method, as previously described, with modifications to enrich DENV (Appendix).

Phylogenetics and Molecular Clock Analysis

To determine specific DENV genotypes, we aligned the Burkina Faso genomes with all complete genomes obtained from the US National Institutes of Health National Institute of Allergy and Infectious Diseases Virus Pathogen Database and Analysis Resource (http://www.viprbrc.org) and inferred a phylogenetic tree by using FastTree 2.1 (https://bioweb.pasteur.fr/packages/pack@FastTree@2.1.10). For our large-scale phylodynamics analysis, we retained all genomes from Africa and randomly subsampled ≈10% of the remaining genomes. We estimated time-calibrated phylogenies with the Markov chain Monte Carlo method implemented in BEAST 1.10.4 (https://beast.community) (Appendix).

Evaluation of PCR Diagnostics

We mapped primers and probe for the CDC Trioplex assay (patent no. WO2018169550A1), CDC DENV-1–4 RT-PCR (*26*), and Johnson et al. DENV RT-PCR (*27*) to the 29 Burkina Faso genomes in Geneious Prime 2021.0.3 (https://www.geneious.com). We then calculated mismatches within the primer–probe binding sites.

We further mapped the Trioplex forward primer, reverse1 primer, and probe sequences to an alignment of all available DENV genomes. We trimmed alignments to each primer–probe region and calculated the number of mismatches. We retained sequences with country information and calculated the proportion of genomes from each country with ≥1 mismatches. We represented these proportions in a chloropleth map by using ArcGIS Pro 2.8.0 (https://pro.arcgis.com).

Vaccine and Epitope Analysis

We compared our Burkina Faso genomes to the Dengvaxia and TetraVax-DV-TV003 vaccine strains through sequence alignment in Geneious Prime 2021.0.3 by using MAFFT 7.427 (https://mafft.cbrc.jp/alignment/software). We were unable to obtain

genome sequences of the TAK-003 dengue vaccine (Takeda, https://www.takeda.com). For the continental comparison, we downloaded all available DENV genomes from the Virus Pathogen Database and Analysis Resource and grouped them by serotype. We aligned the downloaded genomes to the vaccine strains with MAFFT and trimmed them to the membrane precursor (prM) and envelope (E) gene regions; we then retained and translated all genomes with country of origin. We assigned each represented country to a continent and calculated the proportion of sequences with divergent amino acids compared with the vaccines within each continental alignment.

We performed epitope mapping to compare the amino acid diversity of DENV strains from the 2017 outbreak in Burkina Faso to relevant epitopes that could serve as targets for antiviral human monoclonal antibodies. Appropriate epitopes for DENV-1–3 serotypes have been identified previously; we used an approach previously described comparing those amino acid targets and vaccine components to genomes from Burkina Faso (*28*) (Appendix).

Data Availability

We submitted the consensus sequences that we generated from our Burkina Faso samples to GenBank (accession nos. MT261951–79). Probe sequences used during sequencing, nucleotide and amino acid alignments, and the .xml files are available online (https://github.com/cathrnbp/paper-dengue-2021).

Ethics Considerations

The study protocol was approved by the Naval Medical Research Center's Institutional Review Board (project no. NAMRU3.2018.0001). The study was in compliance with all applicable federal regulations governing the protection of human subjects.

Results

Dengue Virus Diversity in Burkina Faso

Only 31 of the 791 samples had a measurable cycle threshold (Ct), and 20 of these met the criteria to be considered positive for the Trioplex assay (Appendix Table 1). Subsequent serologic tests detected NS1 antigen in 44 samples, DENV IgM in 18 samples, and DENV IgG in 27 samples, resulting in a total of 86 samples positive by PCR, NS1 antigen test, IgM test, or all 3 tests; many samples were positive by >1 test (Appendix Table, Figure 1).

We excluded samples positive only for DENV IgG. In total, we describe 29 DENV genomes with >85% coverage from 65 sequenced samples (Table). Genomic analysis confirmed the presence of serotypes 1–3; we identified no mixed serotype infections. To place these 29 genomes in context, we inferred maximum-likelihood and molecular-clock phylogenies for each serotype. Phylogenetic analysis of the genomes classified them into a single genotype for each serotype (Figure 1).

We sequenced 5 DENV-1 genotype V, 16 DENV-2 Cosmopolitan, and 8 DENV-3 genotype III genomes. The DENV-1 genomes grouped closely with a traveler from France returning from Benin in 2019 (GenBank accession no. MN600714) (*29*) and the DENV-2 genomes with a traveler returning to France from Burkina Faso in 2016 (GenBank accession nos. KY627762/3). The DENV-1 genomes have a most recent common ancestor (MRCA) from July 2016 (95% highest posterior density [HPD] 2016.1–2016.9) (Figure 2) and form a monophyletic clade with other genomes from West Africa sampled during 2015–2019, having a common ancestor from September 2014 (95% HPD 2014.0–2015.3). Our analysis of all complete Africa DENV-1 genomes indicates multiple separate introductions into Africa, followed by localized spread (Figure 2). DENV-1 may have been introduced into West Africa as early as 2010 (95% HPD 2009.9–2011.4), probably from Asia. The phylogenetic tree inferred from all E gene sequences corroborates this conclusion (Appendix Figure 2).

Our DENV-2 genomes form several clusters across a monophyletic Africa clade with a MRCA from May 2015 (95% HPD 2014.8–2015.9) (Figure 3). DENV-2 genomes in this clade have been sequenced from countries across West Africa, and available data suggest the 2017 Burkina Faso variant was probably exported to China (Figure 3), demonstrating the movement of DENV from Africa to Asia. In contrast to DENV-1, DENV-2 genomes share a common ancestor with other genomes from Burkina Faso collected as far back as 1983. The MRCA of the entire monophyletic Africa clade, including 2 outlying genomes from Kenya, was from May 1978 (95% HPD 1975.3–1981.1). The long branch from the early 1980s to 2015 is probably the result of undersampling rather than the absence of human DENV-2 cases. To ensure this long branch was not a result of excluded sequencing data in our complete genome analysis, we inferred phylogenetic trees from all E gene sequences from partial and complete genomes (Appendix Figure 3). We identified partial genomes from an additional 9 Africa countries that clustered within the same clade as these Burkina Faso genomes; only genomes sampled from Indonesia in the 1970s were antecedent. These

data demonstrate that DENV-2 has been circulating across Africa since the late 1970s, when it was probably introduced from Southeast Asia.

The molecular-clock phylogeny for DENV-3 genomes from Burkina Faso cluster into 2 distinct clades within a monophyletic Africa clade (Figure 4). The MRCA for the DENV-3 Burkina Faso clade was from January 2013 (95% HPD 2010.8–2014.9) and the MRCA of all Africa genomes from March 2006 (95% HPD 2004.0–2008.1); these genomes were probably introduced from Asia. When including all E gene genomes in a phylogenetic analysis, we see introductions to 8 additional countries in Africa (Appendix Figure 4). These results provide evidence of widespread dengue virus circulation within Africa with DENV-1 existing for >7 years, DENV-2 for >39 years, and DENV-3 for >11 years.

Trioplex Assay in Africa

Although only 31 of the 791 samples we tested were positive by the Trioplex assay, after sequencing we unexpectedly gained complete genomes from 3 samples that were negative by PCR, indicating concerns with PCR sensitivity. The median Trioplex assay Ct value for DENV-1 genomes was 29.5, for DENV-2 was 37.9, and DENV-3 25.3 (Appendix Figure 5), suggesting that the Trioplex assay was less sensitive against DENV-2 than DENV-1 and DENV-3. This finding is corroborated by the limits of detection reported in the Trioplex package insert, which are stated as 5.82×10^4

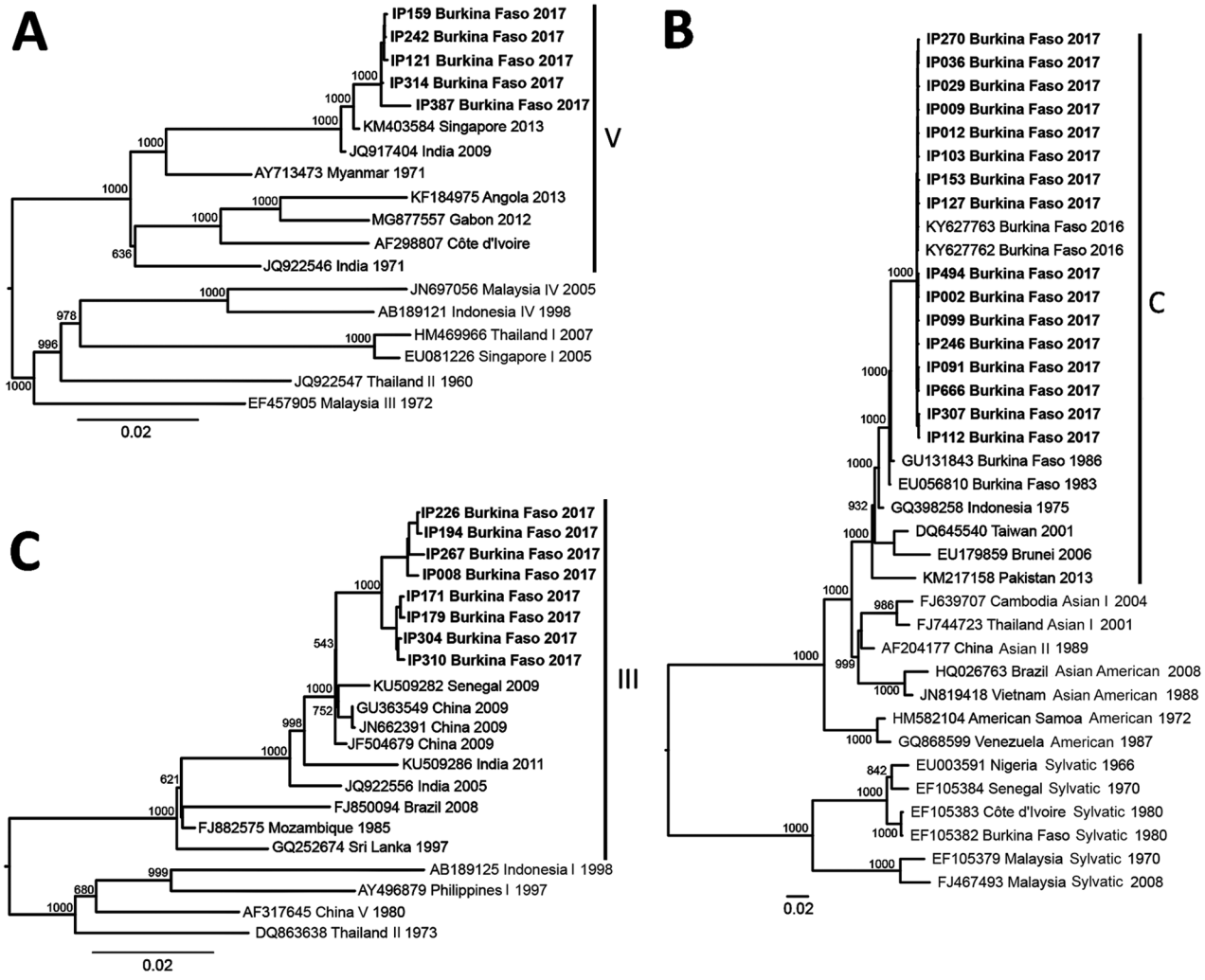


Figure 1. Phylogenetic trees of dengue virus (DENV) serotypes 1 (A), 2 (B), and 3 (C), inferred from an alignment of the 2017 Burkina Faso dengue virus outbreak genomes (boldface) and all other complete genomes from US National Institutes of Health National Institute of Allergy and Infectious Diseases Virus Pathogen Database and Analysis Resource (http://www.viprbrc.org) and pruned to representative genotypes. The Burkina Faso genomes were DENV-1 genotype V, DENV-2 genotype Cosmopolitan, and DENV-3 genotype III. GenBank accession numbers are provided for reference genomes.

Table. Suspected dengue virus–positive samples from the 2017 Burkina Faso dengue virus outbreak, found to be positive by CDC Trioplex real-time RT-PCR or serologic testing, and sequencing results for samples that generated genomes with >85% coverage*

NMIMR laboratory ID	Specimen collection date, 2017	PCR results, Ct	Serologic results			Sequencing results		
			NS1 Ag	IgM	IgG	Serotype	Genome coverage, %	GenBank accession no.
IP-002	Oct 16	UND	+	–	–	DENV-2	99.6	MT261956
IP-008	Oct 16	35.5	+	–	–	DENV-3	99.1	MT261972
IP-009	Oct 16	37.1	+	–	–	DENV-2	99.7	MT261957
IP-012	Oct 16	40.6	+	–	–	DENV-2	87.6	MT261958
IP-029	Oct 17	37.5	+	–	–	DENV-2	98.8	MT261959
IP-036	Oct 17	40.6	+	–	–	DENV-2	99.5	MT261960
IP-091	Oct 26	36	+	–	–	DENV-2	99.8	MT261961
IP-099	Oct 25	40.3	+	–	–	DENV-2	99.6	MT261962
IP-103	Oct 25	34.6	+	–	–	DENV-2	99.7	MT261963
IP-112	Oct 24	39.5	+	–	–	DENV-2	99.7	MT261964
IP-121	Oct 23	29	+	–	–	DENV-1	99.7	MT261951
IP-127	Oct 23	38.2	–	–	–	DENV-2	99.4	MT261965
IP-153	Nov 8	33.7	+	–	–	DENV-2	99.8	MT261966
IP-159	Nov 9	32	+	–	–	DENV-1	99.3	MT261952
IP-171	Nov 9	33.8	+	+	+	DENV-3	94.3	MT261973
IP-179	Nov 13	22.5	+	–	–	DENV-3	94.4	MT261974
IP-194	Nov 17	25.2	–	–	–	DENV-3	99.7	MT261975
IP-226	Oct 4	25.4	–	–	–	DENV-3	99.8	MT261976
IP-242	Oct 9	29.5	–	–	–	DENV-1	99.6	MT261953
IP-246	Oct 9	31.1	–	–	–	DENV-2	99.8	MT261967
IP-267	Oct 12	19.3	–	–	–	DENV-3	99.8	MT261977
IP-270	Oct 12	37.1	–	–	–	DENV-2	99.5	MT261968
IP-304	Oct 27	24.1	+	–	–	DENV-3	99.7	MT261978
IP-307	Oct 30	37.3	–	–	–	DENV-2	99.7	MT261969
IP-310	Nov 2	30.3	+	–	+	DENV-3	95.7	MT261979
IP-314	Nov 2	23.8	+	–	–	DENV-1	99.7	MT261954
IP 387	Dec 12	UND	+	–	–	DENV-1	88.4	MT261955
IP 494	Nov 3	41.2	+	–	–	DENV-2	99.6	MT261970
IP 666	Nov 6	UND	+	–	–	DENV-2	99.6	MT261971

*CDC, US Centers for Disease Control Prevention; Ct, cycle threshold; NMIMR, Noguchi Memorial Institute for Medical Research; NS1 Ag, nonstructural protein 1 antigen; RT-PCR, reverse transcription PCR; UND, undetected; +, positive; –, negative.

genome copies/mL for DENV-1, 8.25×10^4 genome copies/mL for DENV-2, and 4.36×10^4 genome copies/mL for DENV-3.

In addition, we performed an in silico analysis of these assays by mapping the primers and probe to the Burkina Faso genomes and comparing nucleotide homogeneity. The Trioplex primers and probe were identical to the DENV-1 and DENV-3 Burkina Faso genomes, but both the forward and reverse1 primers had a single mismatch when mapped to the DENV-2 genomes. We also investigated the CDC DENV-1–4 RT-PCR (*26*), which had 5 mismatches across the primers and probe for the DENV-1, DENV-2, and DENV-3, and the Johnson et al. RT-PCR (*27*), which had 8 mismatches (Appendix Figure 6).

To determine if these mismatches were specific to Burkina Faso or indicated a more global problem, we mapped the Trioplex primers and probe to all available DENV genomes and calculated the proportion of genomes from each country that exhibited <100% homogeneity to the primers and probe (i.e., had ≥1 mismatch) (Figure 5). Because the Trioplex assay targets the 5′ untranslated region and many genomes lacked coverage in this region, especially for the forward primer, they could not be included. For DENV-1 and DENV-3, we observed almost complete homogeneity between the probe and reverse1 primer within all countries. The forward primer was similarly identical, except for some divergence in Asia and North America. Conversely, for DENV-2, although the probe sequence was almost completely identical to the DENV-2 genomes at its binding site, the forward primer exhibited a single mismatch in every genome included in our analysis. This mismatch is likely the cause of the lowered limit of detection for DENV-2 compared with DENV-1 and DENV- 3, as noted previously. Approximately 95% of genomes from Africa had ≥1 mismatches in the reverse1 primer (and a mismatch in the forward primer) compared with 6% of genomes from South America, 20% from Oceania, and 50% from Asia (Figure 5).

Dengue Vaccines and African Variants

The 29 full genomes from the Burkina Faso 2017 outbreak were compared with the Dengvaxia and TetraVax-DV-TV003 vaccine strains for each serotype (Figure 6). Dengvaxia is based on an immunoprotective serotype-specific prM and E gene region in a

background of yellow fever virus while TetraVax-DV-TV003 uses a different dengue virus serotype. Therefore, the comparison with the full genome sequences focused on the prM and E proteins. Divergent amino acids occurred throughout the prM and E proteins between the vaccine strain and Burkina Faso wild types,

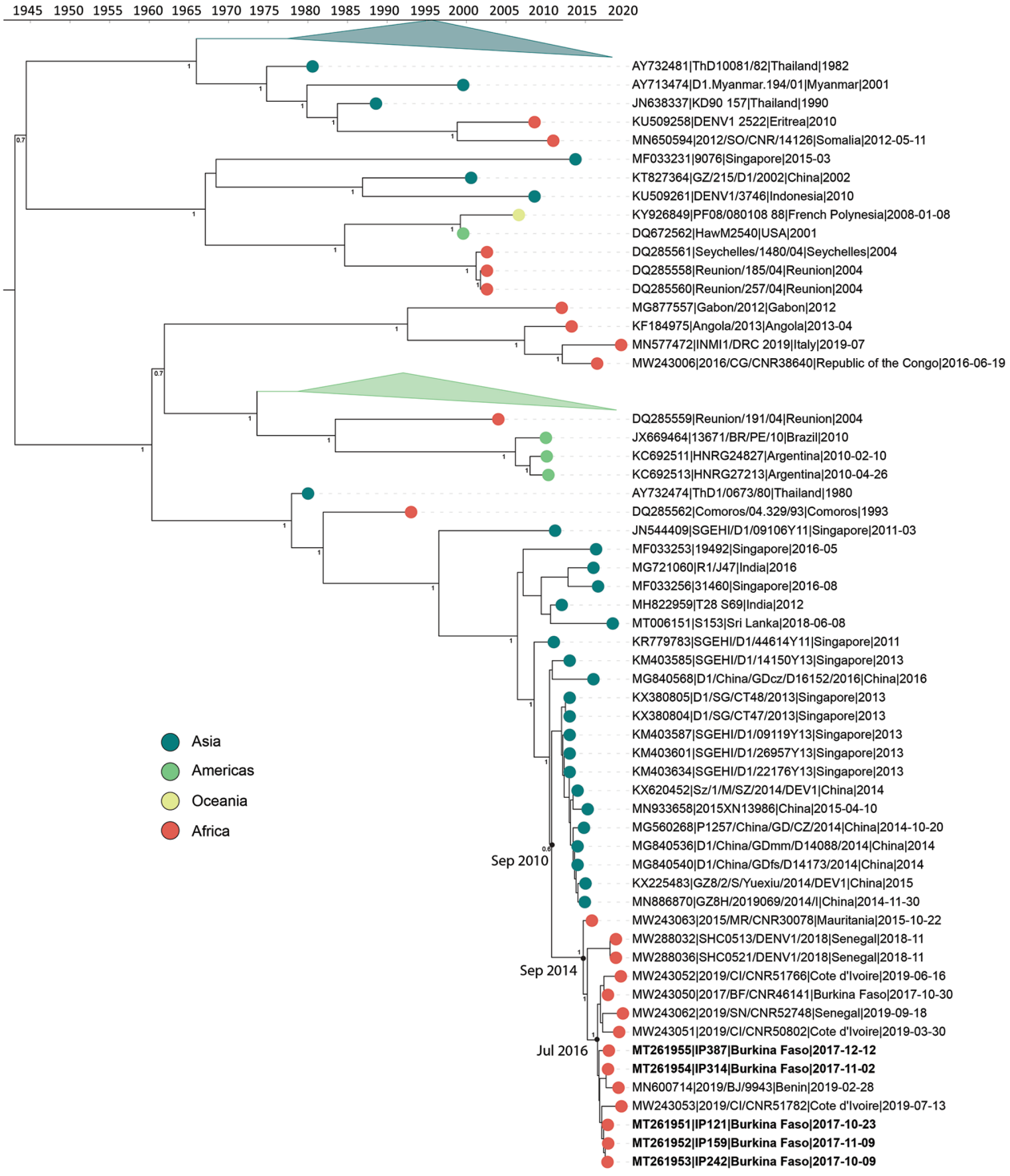


Figure 2. Time-calibrated phylogenetic trees of a subset of global dengue virus 1 genomes and 2017 Burkina Faso dengue virus outbreak genomes (boldface). Colored circles indicate geographic origin. Dates indicate the most recent common ancestor for the 2017 Burkina Faso dengue virus outbreak and all genomes from Africa. Posterior probabilities are indicated at major nodes. GenBank accession numbers are provided for reference genomes.

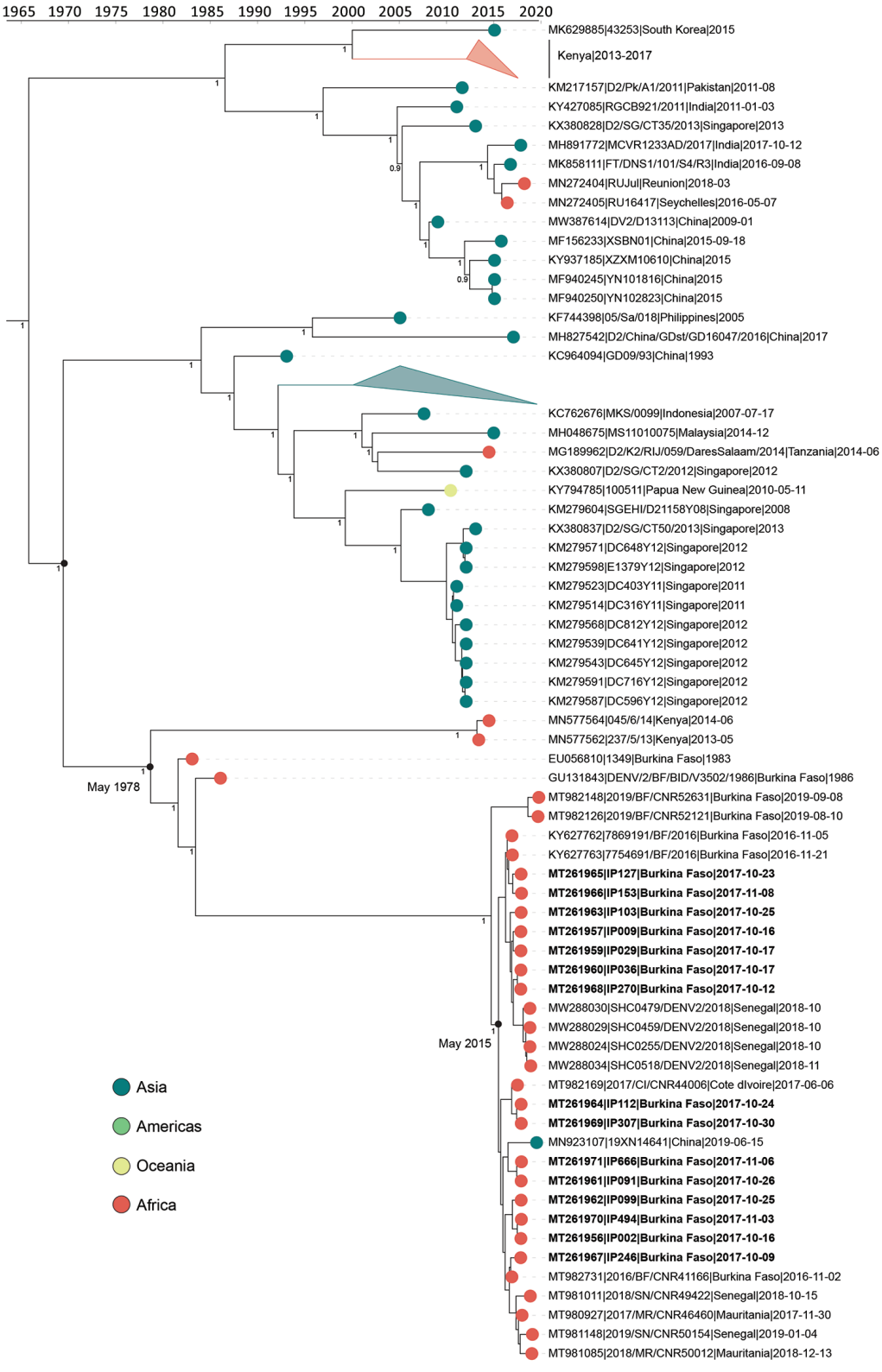


Figure 3. Time-calibrated phylogenetic trees of a subset of global dengue virus 2 genomes and 2017 Burkina Faso dengue virus outbreak genomes (boldface). Colored circles indicate geographic origin. Dates indicate the most recent common ancestor for the 2017 Burkina Faso dengue virus outbreak and all genomes from Africa. Posterior probabilities are indicated at major nodes. GenBank accession numbers are provided for reference genomes.

including 20 substitutions for DENV-1 sequences, 18 for DENV-2, and 17 for DENV-3 when compared with the Dengvaxia vaccine and 18 substitutions for DENV-1, 25 for DENV-2, and 19 for DENV-3 when compared with the TetraVax-DV-TV003 vaccine. None of the discordant amino acids clustered to any particular structural domain.

We compared the Burkina Faso wild type virus sequences with the vaccine strains at 8 B cell epitopes (Figure 7). The noted divergence is similar to that

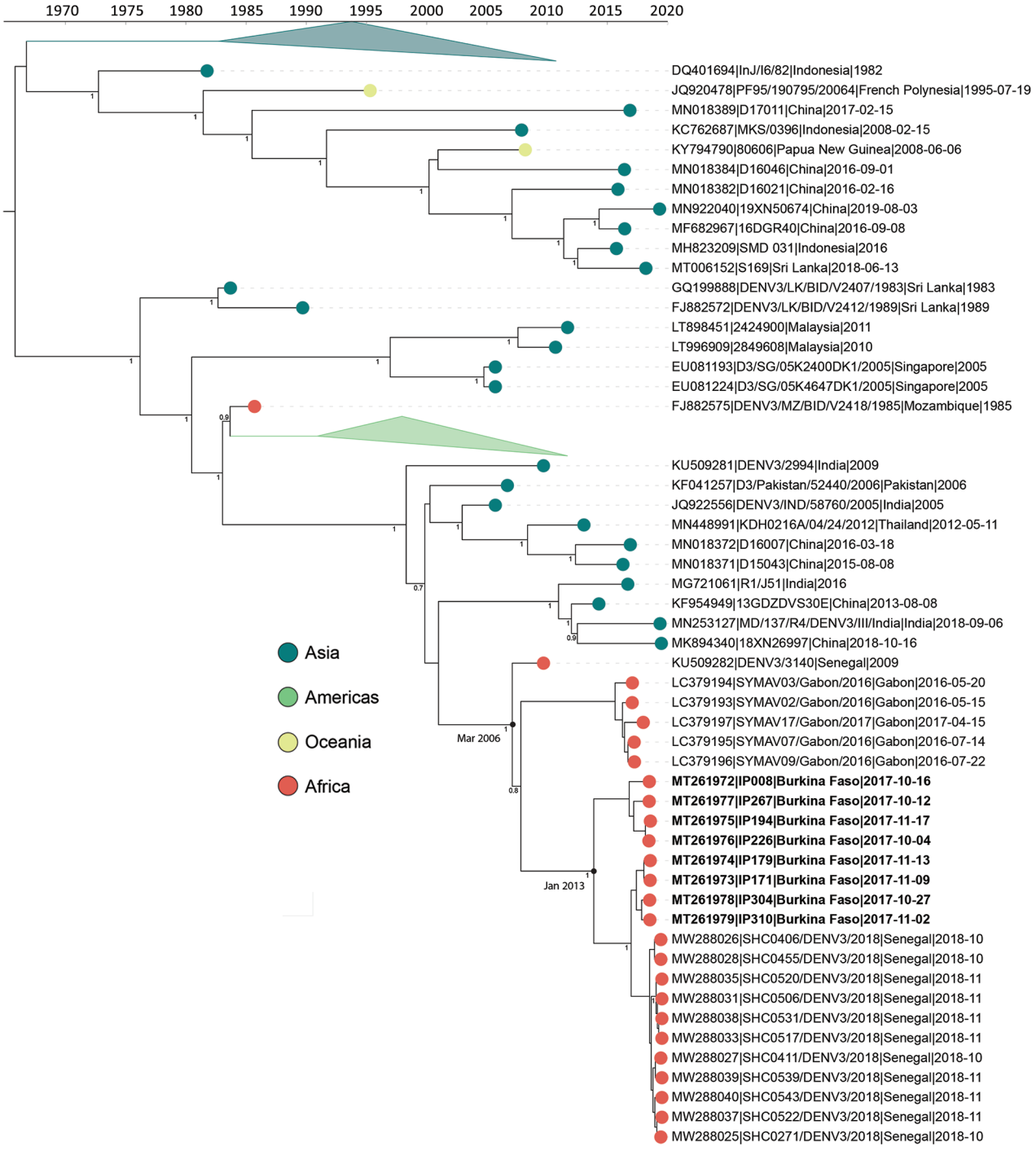


Figure 4. Time-calibrated phylogenetic trees of a subset of global dengue virus 3 genomes and 2017 Burkina Faso dengue virus outbreak genomes indicated (boldface). Colored circles indicate geographic origin. Dates indicate the most recent common ancestor for the 2017 Burkina Faso dengue virus outbreak and all genomes from Africa. Posterior probabilities are indicated at major nodes. GenBank accession numbers are provided for reference genomes.

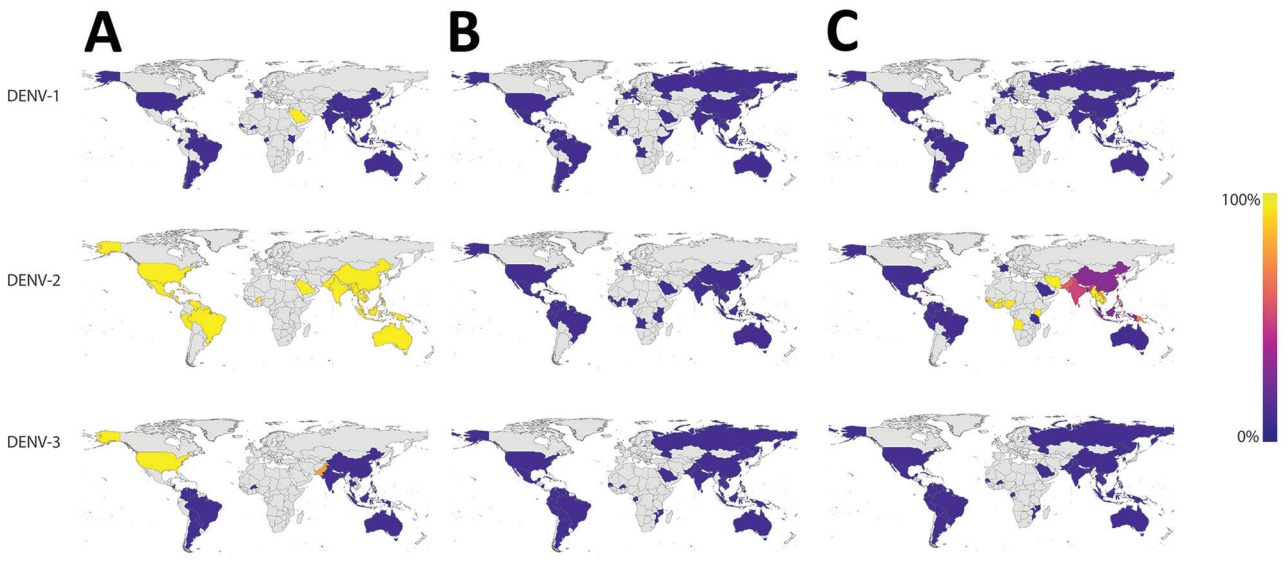


Figure 5. Nucleotide identity between dengue virus molecular diagnostics and all sequenced DENV genomes from the 2017 Burkina Faso dengue outbreak. The map indicates the proportion of genomes from each country with ≥1 mismatches against the Trioplex PCR forward primer (A), probe (B), and reverse1 primer (C). Countries in gray have no data. DENV-1 and DENV-3 have concordant nucleotide identity to the primers and probe, but most DENV-2 forward primer and reverse1 primer in sequences from Africa have a high proportion of genomes with ≥1 mismatches to the Trioplex PCR's primers and probe. DENV-1, dengue virus serotype 1; DENV-2, dengue virus serotype 2; DENV-3, dengue virus serotype 3

seen in Southeast Asia and the Americas and has been previously described at E protein sites 155, 161, and 171 for DENV-1; sites 71 and 149 for DENV-2; and site 124 for DENV-3 (*28*).

Because of the paucity of genomic data from Burkina Faso, we expanded our analysis to the continental scale. We calculated the proportion of genomes within each continental alignment diverging from the vaccine sequence at each amino acid position. Amino acid positions with >5% divergence from the Dengvaxia (Appendix Figure 7) and TetraVax-DV-TV003 (Appendix Figure 8) vaccine strains were retained. In a minimum of 12 amino acid positions across each serotype and vaccine comparison, DENV genomes from Africa had the greatest proportion of genomes divergent from the vaccine strains. DENV genomes circulating in Africa exhibit their own genomic diversity, impairing the potential effectiveness of a DENV vaccine on that continent.

Discussion

We sequenced 29 full DENV genomes from the 2017 outbreak in Burkina Faso, confirming cocirculation of DENV-1, DENV-2, and DENV-3 serotypes. Phylogenetic analysis of DENV-2 genomes show the most similar genomes to those from the DENV 2017 outbreak are also from Burkina Faso, dating from 1983 through 1986. The genetic similarities between DENV-2 strains from 2017 and those from >30 years ago suggest local circulation of DENV-2 genotype Cosmopolitan both within Burkina Faso and in other countries in West Africa and that DENV-2 is endemic to this area. All the genomes from the 2017 outbreak in Burkina Faso were most closely related to strains from Africa or Asia and not those from the Americas. This finding could be attributable to greater trade, travel, and economic-based contact between Burkina Faso and other countries of Africa with Asia as opposed to countries in the Americas.

We obtained 2 complete genomes and 1 partial genome from PCR-negative samples, and the Ct for DENV-2 samples was consistently higher than that for DENV-1 and DENV-3, suggesting a drop in assay sensitivity against DENV-2 genomes. This decrease is probably because of mismatches between the primers and probe and target sequences, or because the samples were too degraded for PCR but not for hybrid capture sequencing, which seems unlikely. An in silico analysis identified mismatches between the primers and probe for the Trioplex assay and DENV-2 genomes, both in our Burkina Faso genomes and across Africa. The Trioplex assay was designed during the 2015–2016 Zika virus epidemic to differentiate between Zika, chikungunya, and DENV infections and has also been made available to international laboratories in a lyophilized format at no charge (*30*). This altruism means that it is a commonly used assay in low-resource laboratories, such as those in many

countries in Africa. The Trioplex assay was validated by using samples collected in Puerto Rico (*30*). In our analyses, genomes from the Americas were most congruent with the Trioplex primers and probe and those from Africa were the least congruent. Further, the target of the Trioplex assay is near the 5′ untranslated region and vulnerable to degradation, which is more likely to occur in low-resource countries, where samples are often transported to a central laboratory under less than ideal conditions for RNA preservation. The CDC developed another PCR with serotype-specific primers and probe, the CDC-DENV-1–4 RT-PCR (*26*), based on the Johnson et al. RT-PCR (*27*), but both of these assays exhibited even less nucleotide homogeneity in silico than the Trioplex assay. The observed genomic divergence, discordance between sequencing and PCR results, and existence of multiple mismatches in the primer binding site within samples from Africa suggest that Africa-specific virus evolution is occurring, probably leading to an underreporting of dengue cases because of insensitive diagnostics. This probability necessitates the development of diagnostics that account for the unique molecular divergence occurring in Africa to have an accurate assessment of the disease burden of DENV and improve patient care.

Because of the threat that DENV poses to Africa, the number of outbreaks, and the lack of countermeasures, it is not too early to consider preventive measures. The Burkina Faso genomes enabled us to perform in silico analyses of DENV vaccine efficacy and assess divergence from known important epitopes. In general, the 3 DENV serotypes circulating during the 2017 outbreak in Burkina Faso were very similar to the vaccine strains used in the CYD- Dengvaxia and TetraVax-DV-TV003 vaccines.

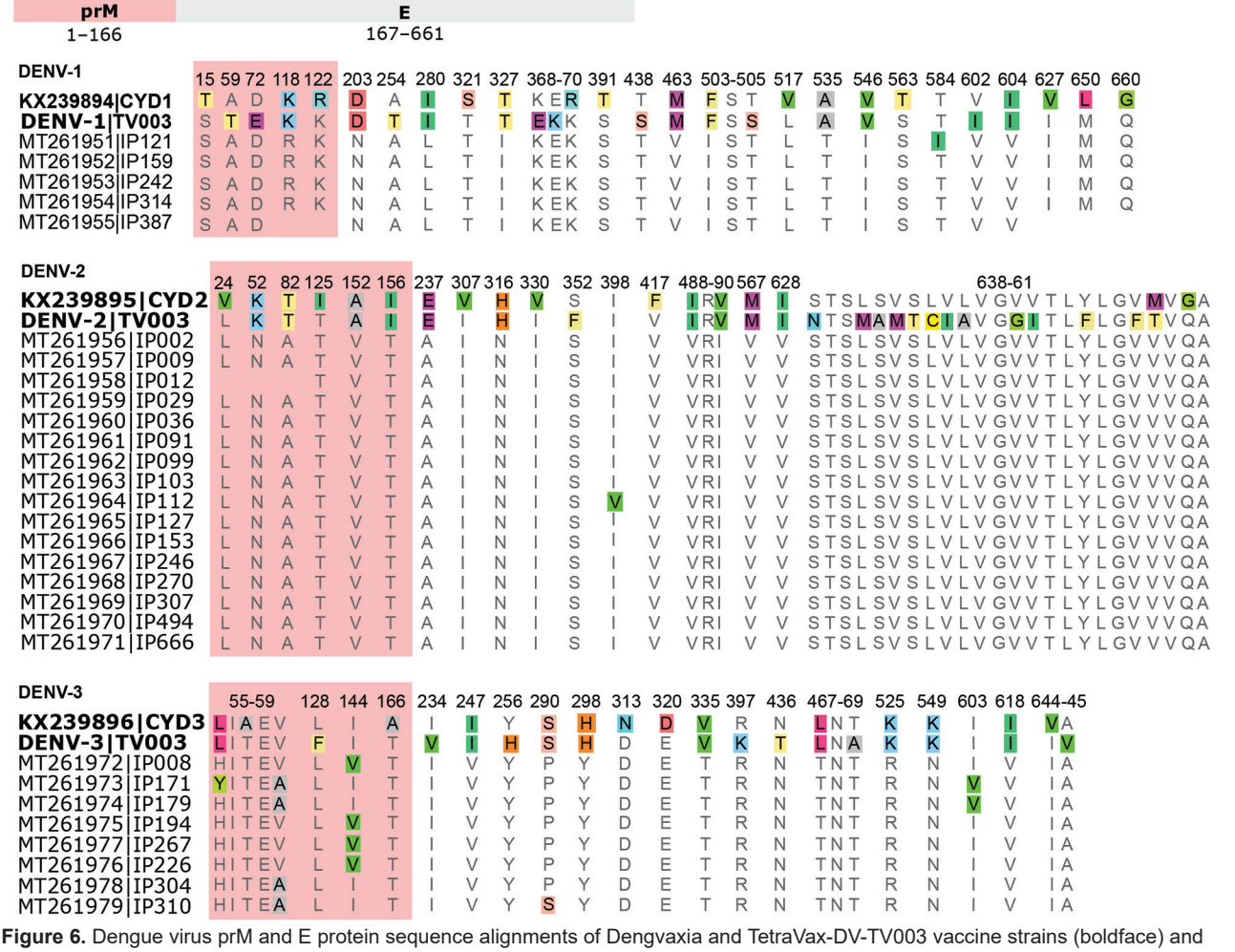


Figure 6. Dengue virus prM and E protein sequence alignments of Dengvaxia and TetraVax-DV-TV003 vaccine strains (boldface) and 2017 Burkina Faso dengue virus outbreak genomes for serotypes 1, 2, and 3. Only amino acid positions with disagreements are shown; single-point disagreements are highlighted. For clarity, prM protein sequences are shaded in red. Numerals represent the prM and E protein amino acid position. CYD, Dengvaxia vaccine; DENV-1, dengue virus serotype 1; DENV-2, dengue virus serotype 2; DENV-3, dengue virus serotype 3; E, envelope; prM, premembrane; TV003, TetraVax-DV-TV003 vaccine.

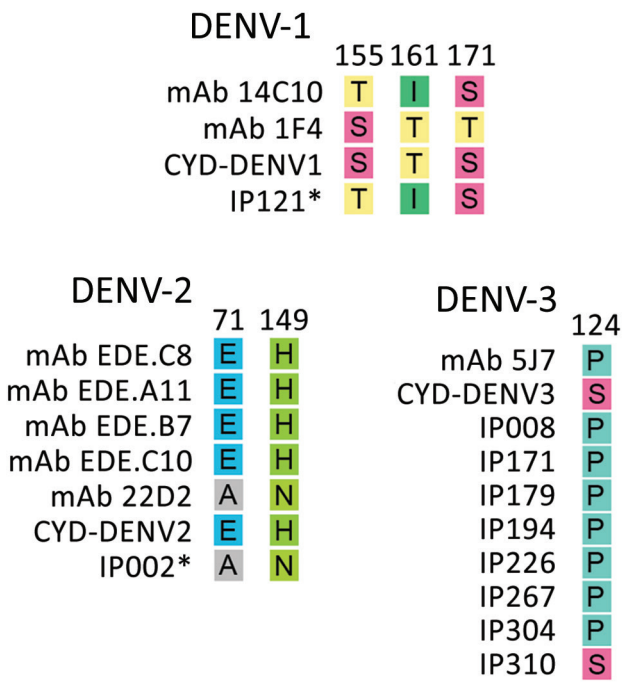


Figure 7. Amino acid mismatch comparison between 2017 Burkina Faso dengue virus outbreak genomes and virus neutralizing human mAbs for the 3 dengue virus serotypes. The amino acid changes presented are expected to disrupt binding between the envelope protein and heavy chain of the monoclonal antibodies. Dengvaxia vaccine amino acid included for comparison. Asterisk indicates all of the 2017 Burkina Faso dengue virus outbreak genomes share the same amino acid at that position. Numerals represent the E protein amino acid position. CYD, Dengvaxia vaccine; DENV-1, dengue virus serotype 1; DENV-2, dengue virus serotype 2; DENV-3, dengue virus serotype 3; E, envelope; mAb, monoclonal antibody.

Although the Dengvaxia vaccine was noted to have decreased efficacy against DENV-2 compared with other serotypes (*31*), it appears to have been more efficacious against the DENV-2 Cosmopolitan genotype than against the Asian 1 genotype (*28*). However, there were key positions in the Dengvaxia and TetraVax-DV-TV003 vaccine sequences where genomes from Africa diverged more often than genomes from other continents, indicating the development of unique diversity within Africa. Further research is needed to understand how various genotypes and subtle differences at the amino acid level of prM and E proteins affect clinical immunity. Additional in vivo testing is necessary to determine if a dengue vaccine could be used in West Africa.

The amino acid prM and E protein sequences from the Burkina Faso DENV outbreak were also very similar to known targets for B cell epitopes. The differences noted have been previously reported in DENV strains from the Americas and Southeast Asia (*28*). However, we observed 2 mismatches at important epitope sites E71 and E149 among all DENV-2 Cosmopolitan samples. Although this discordance is documented in other DENV-2 genotypes, including American, American-Asian, Asian 1, and Asian II genotypes, it is not as well defined in the Cosmopolitan genotype.

A limitation of our study is that ≥1 year had passed since the initial collection of the samples before next-generation sequencing was performed, introducing multiple factors that could have contributed to this low percentage of positive results: sample degradation over time, less than ideal storage, low viremia, poor coverage of the assay, or a combination of these factors. Using further molecular diagnostics may have revealed more DENV-positive samples but were not available in the country at the time of the study. Additional genomes could have increased the probability of detecting unusual genomes or amino acid changes. Assessing the evolutionary patterns of DENV is difficult because so few whole DENV genomes from Africa are available on GenBank to compare with the genomes from Burkina Faso. Finally, donor virus strains other than Dengvaxia and TetraVax-DV-TV003 were not assessed.

Our assessment of DENV whole genomes from Burkina Faso provide information on the molecular epidemiology of this virus and divergence from diagnostics, vaccine strains, and B cell epitopes. Further surveillance of contemporary DENV genotypes in Africa is needed to address the contemporary antigenic and genetic variations within a region. The endemicity of DENV and increasing number of outbreaks in countries like Burkina Faso suggest the need for the development of diagnostics that account for ongoing viral evolution in Africa and consideration for adding countries in Africa to DENV clinical trials to address the emerging public health threat.

Acknowledgments

We thank Steve Whitehead for sharing TetraVax-DV-TV003 genome sequences.

This work was funded by the Armed Forces Health Surveillance Branch and its Global Emerging Infections Surveillance and Response Section (work unit no. ProMIS ID P0142_19_N3). The funder played no role in the design of the study, sample collection, analysis and interpretation of results, or manuscript preparation.

Genomes have been submitted to GenBank. Individual participant data beyond what is available in GenBank will not be available.

All authors declare no competing or conflicts of interest. A.L. and N.D. are military service members or government employees. This work was prepared as part of their official duties. Title 17, U.S.C., §105 provides that copyright protection under this title is not available for any work of the US government. Title 17, U.S.C., §101 defines a US government work as a work prepared by a military service member or employee of the US government as part of that person's official duties. The views expressed in the article are those of the authors and do not necessarily express the official policy and position of the US Navy, the US Department of Defense, the US government, or the institutions affiliated with the authors.

About the Author

Dr. Letizia is the science director at the Naval Medical Research Unit TWO in Singapore and is a board-certified infectious disease physician on active duty in the US Navy. His research interest is in emerging infectious diseases, including dengue virus.

References

1. Simmons CP, Farrar JJ, Nguyen VVC, Wills B. Dengue. N Engl J Med. 2012;366:1423–32. https://doi.org/10.1056/NEJMra1110265
2. Brady OJ, Gething PW, Bhatt S, Messina JP, Brownstein JS, Hoen AG, et al. Refining the global spatial limits of dengue virus transmission by evidence-based consensus. PLoS Negl Trop Dis. 2012;6:e1760. https://doi.org/10.1371/journal.pntd.0001760
3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. Nature. 2013;496:504–7. https://doi.org/10.1038/nature12060
4. Guzman MG, Harris E. Dengue. Lancet. 2015;385:453–65. https://doi.org/10.1016/S0140-6736(14)60572-9
5. Weetman D, Kamgang B, Badolo A, Moyes CL, Shearer FM, Coulibaly M, et al. *Aedes* mosquitoes and *Aedes*-borne arboviruses in Africa: current and future threats. Int J Environ Res Public Health. 2018;15:E220. https://doi.org/10.3390/ijerph15020220
6. Lim JK, Carabali M, Lee JS, Lee KS, Namkung S, Lim SK, et al. Evaluating dengue burden in Africa in passive fever surveillance and seroprevalence studies: protocol of field studies of the Dengue Vaccine Initiative. BMJ Open. 2018;8:e017673. https://doi.org/10.1136/bmjopen-2017-017673
7. Stoler J, Al Dashti R, Anto F, Fobil JN, Awandare GA. Deconstructing "malaria": West Africa as the next front for dengue fever surveillance and control. Acta Trop. 2014;134:58–65. https://doi.org/10.1016/j.actatropica.2014.02.017
8. Fournet F, Rican S, Vaillant Z, Roudot A, Meunier-Nikiema A, Kassié D, et al. The influence of urbanization modes on the spatial circulation of flaviviruses within Ouagadougou (Burkina Faso). Int J Environ Res Public Health. 2016;13:E1226. https://doi.org/10.3390/ijerph13121226
9. Schwartz E, Meltzer E, Mendelson M, Tooke A, Steiner F, Gautret P, et al. Detection on four continents of dengue fever cases related to an ongoing outbreak in Luanda, Angola, March to May 2013. Euro Surveill. 2013;18:20488. https://doi.org/10.2807/ese.18.21.20488-en
10. Toro C, Trevisi P, López-Quintana B, Amor A, Iglesias N, Subirats M, et al. Imported dengue infection in a Spanish hospital with a high proportion of travelers from Africa: a 9-year retrospective study. Am J Trop Med Hyg. 2017;96:701–7. https://doi.org/10.4269/ajtmh.16-0335
11. Pollett S, Melendrez MC, Maljkovic Berry I, Duchêne S, Salje H, Cummings DAT, et al. Understanding dengue virus evolution to support epidemic surveillance and countermeasure development. Infect Genet Evol. 2018;62:279–95. https://doi.org/10.1016/j.meegid.2018.04.032
12. Malisheni M, Khaiboullina SF, Rizvanov AA, Takah N, Murewanhema G, Bates M. Clinical efficacy, safety, and immunogenicity of a live attenuated tetravalent dengue vaccine (CYD-TDV) in children: a systematic review with meta-analysis. Front Immunol. 2017;8:863. https://doi.org/10.3389/fimmu.2017.00863
13. Eltom K, Enan K, El Hussein ARM, Elkhidir IM. Dengue virus infection in sub-Saharan Africa between 2010 and 2020: a systematic review and meta-analysis. Front Cell Infect Microbiol. 2021;11:678945. https://doi.org/10.3389/fcimb.2021.678945
14. Vanneste K, Garlant L, Broeders S, Van Gucht S, Roosens NH. Application of whole genome data for in silico evaluation of primers and probes routinely employed for the detection of viral species by RT-qPCR using dengue virus as a case study. BMC Bioinformatics. 2018;19:312. https://doi.org/10.1186/s12859-018-2313-0
15. Sessions OM, Wilm A, Kamaraj US, Choy MM, Chow A, Chong Y, et al. Analysis of dengue virus genetic diversity during human and mosquito infection reveals genetic constraints. PLoS Negl Trop Dis. 2015;9:e0004044. https://doi.org/10.1371/journal.pntd.0004044
16. Amarasinghe A, Kuritsk JN, Letson GW, Margolis HS. Dengue virus infection in Africa. Emerg Infect Dis. 2011;17:1349–54.
17. Katzelnick LC, Coloma J, Harris E. Dengue: knowledge gaps, unmet needs, and research priorities. Lancet Infect Dis. 2017;17:e88–100. https://doi.org/10.1016/S1473-3099(16)30473-X
18. Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics of dengue virus. Infect Genet Evol. 2003;3:19–28. https://doi.org/10.1016/S1567-1348(03)00004-2
19. Usme-Ciro JA, Méndez JA, Laiton KD, Páez A. The relevance of dengue virus genotypes surveillance at country level before vaccine approval. Hum Vaccin Immunother. 2014;10:2674–8. https://doi.org/10.4161/hv.29563
20. Gonzalez JP, Du Saussay C, Gautun JC, McCormick JB, Mouchet J. Dengue in Burkina Faso (ex-Upper Volta): seasonal epidemics in the urban area of Ouagadougou [in French]. Bull Soc Pathol Exot. 1985;78:7–14.
21. Tarnagda Z, Cissé A, Bicaba BW, Diagbouga S, Sagna T, Ilboudo AK, et al. Dengue fever in Burkina Faso, 2016. Emerg Infect Dis. 2018;24:170–2. https://doi.org/10.3201/eid2401.170973
22. World Health Organization. Dengue fever—Burkina Faso. 2016 [cited 2021 Aug 25]. https://www.who.int/emergencies/disease-outbreak-news/item/18-november-2016-dengue-burkina-faso-en
23. World Health Organization. Dengue fever—Burkina Faso. 2017 [cited 2021 Aug 25]. https://www.who.int/emergencies/disease-outbreak-news/item/6-november-2017-dengue-burkina-faso-en
24. Hashimoto T, Kutsuna S, Maeki T, Tajima S, Takaya S, Katanami Y, et al. A case of dengue fever imported from

Burkina Faso to Japan in October 2016. Jpn J Infect Dis. 2017;70:675–7. https://doi.org/10.7883/yoken.JJID.2017.181
25. Baronti C, Piorkowski G, Touret F, Charrel R, de Lamballerie X, Nougairede A. Complete coding sequences of two dengue virus type 2 strains isolated from an outbreak in Burkina Faso in 2016. Genome Announc. 2017;5:e00209–17. https://doi.org/10.1128/genomeA.00209-17
26. Santiago GA, Vergne E, Quiles Y, Cosme J, Vazquez J, Medina JF, et al. Analytical and clinical performance of the CDC real time RT-PCR assay for detection and typing of dengue virus. PLoS Negl Trop Dis. 2013;7:e2311. https://doi.org/10.1371/journal.pntd.0002311
27. Johnson BW, Russell BJ, Lanciotti RS. Serotype-specific detection of dengue viruses in a fourplex real-time reverse transcriptase PCR assay. J Clin Microbiol. 2005;43:4977–83. https://doi.org/10.1128/JCM.43.10.4977-4983.2005
28. Rabaa MA, Girerd-Chambaz Y, Duong Thi Hue K, Vu Tuan T, Wills B, Bonaparte M, et al. Genetic epidemiology of dengue viruses in phase III trials of the CYD tetravalent dengue vaccine and implications for efficacy. eLife. 2017;6:e24196. https://doi.org/10.7554/eLife.24196
29. Fourié T, Luciani L, Amrane S, Zandotti C, Leparc-Goffart I, Ninove L, et al. Dengue virus type 1 infection in traveler returning from Benin to France, 2019. Emerg Infect Dis. 2020;26:1946–9. https://doi.org/10.3201/eid2608.200055
30. Santiago GA, Vázquez J, Courtney S, Matías KY, Andersen LE, Colón C, et al. Performance of the Trioplex real-time RT-PCR assay for detection of Zika, dengue, and chikungunya viruses. Nat Commun. 2018;9:1391. https://doi.org/10.1038/s41467-018-03772-1
31. Hadinegoro SR, Arredondo-García JL, Capeding MR, Deseda C, Chotpitayasunondh T, Dietze R, et al.; CYD-TDV Dengue Vaccine Working Group. Efficacy and long-term safety of a dengue vaccine in regions of endemic disease. N Engl J Med. 2015;373:1195–206. https://doi.org/10.1056/NEJMoa1506223

Address for correspondence: Andrew Letizia, PSA Sembawang, Deptford Rd. Building 7-4, 759657, Singapore; email: andrew.g.letizia.mil@mail.mil; Catherine B. Pratt, Rm 4043 Wittson Hall, 984388 Nebraska Medical Center, Omaha, NE 68198-4388, USA; email: catherine.pratt@unmc.edu; Lassana Sangaré, CHU Yalgado Ouédraogo (CHU-YO), 03 BP 7022 Ouagadougou 03, Burkina Faso; email: sangarelassana01@gmail.com

Article DOI: https://doi.org/10.3201/eid2806.212491

Retrospective Genomic Characterization of a 2017 Dengue Virus Outbreak, Burkina Faso

Appendix

Supplemental Methods

Next-generation sequencing (NGS) used the KAPA RNA HyperPrep library preparation kit (KAPA Biosystems, MA), followed by the RNA Enrichment component of the TruSeq RNA Exome kit (Illumina, CA) with 76,380 dengue probes (https://github.com/cathrnbp/paper-dengue-2021). Sixty-five samples were barcoded, pooled and sequenced using the 600-cycle Miseq Reagent kits v3 (Illumina, CA) on an Illumina Miseq with a minimum of 2 × 151-bp reads. For data analysis, the sequence of random hexamer associated with read one and the Illumina adaptors were removed from the sequencing reads using Cutadapt v1.9.dev1, and low-quality reads or bases were filtered using Prinseq-lite v0.20.3. Reads were de novo assembled into contigs using Ray2 and aligned to the NCBI database using BLAST. When viral contigs were detected, reads were aligned to a reference genome using Bowtie2 v2.0.6, duplicates were removed with Picard (http://broadinstitute.github.io/picard), and a new consensus was generated using a combination of Samtools v0.1.18 and custom scripts (https://github.com/jtladner/Scripts/blob/master/reference-based_assembly/consensus_fasta.py). Only bases with Phred quality score ≥20 were used in consensus calling, and a minimum of 3× read-depth coverage, in support of the consensus, were required to make a call; positions lacking this depth of coverage were treated as missing.

Detailed Phylogenetics and Molecular Clock Analysis

Dengue virus (DENV) genomes longer than 10,000 bp were obtained from the NIAID Virus Pathogen Database and Analysis Resource at http://www.viprbrc.org (DENV1 n=2671; DENV2 n=2091; DENV3 n=1102) (*1*). To determine specific DENV genotypes, genomes were first aligned using MAFFT v7.427 (*2*). Maximum-likelihood phylogenetic trees were

exhaustively (-slow) estimated using FastTree v2.1 with 5000 Shimodaira-Hasegawa tests to compare alternate topologies (*3*). Subtree's were selected and pruned using Figtree and Inkscape.

For our large scale phylodynamics analysis, all genomes from Africa were retained and approximately 10% of the remaining genomes were randomly subsampled. These subsets and our novel genomes from Burkina Faso were aligned using MAFFT v7.427 (DENV1 n=292; DENV2 n=281; DENV3 n=134). The alignment was manually verified in Geneious Prime 2021.0.3 (https://www.geneious.com) and trimmed to the coding sequence. We inferred the maximum-likelihood phylogeny with RAxML v8.2.12 and visualized the tree with TempEst v1.5.3 (*4*). Clock-likeness was assessed with the root to tip panel and outliers were removed from the alignment and in the case of DENV2, genomes from the same country and year were further randomly down-sampled. Final subset alignments consisted of 202, 265, and 133 genomes for DENV1, 2, and 3, respectively.

Time-calibrated phylogenies were estimated with the MCMC method implemented in BEAST v1.10.4 (*5*). The GTR+Γ nucleotide substitution model and Bayesian Skyride time-aware model were used in six independent chains for 50 million steps. Tracer v1.7.1 was used to check convergence of individual and combined chains after removing 10% burn-in (*6*). Tree files were combined with Logcombiner v1.10.4 and a maximum clade credibility tree was generated using TreeAnnotator v1.10.4.

As much of the historic genomic data for DENV consists of partial genomes, and to ensure our phylogenetic trees were not missing crucial data, we also downloaded all DENV genomes from ViPR (DENV1=10,896; DENV2=8,800; DENV3=5,929). Genomes were split by serotype and aligned with MAFFT. Alignments were manually verified in Geneious Prime 2021.0.3, trimmed to the E-gene sequence, and phylogenetic tree inferred using FastTree v2.1.12.

References

1. Pickett BE, Sadat EL, Zhang Y, Noronha JM, Squires RB, Hunt V, et al. ViPR: an open bioinformatics database and analysis resource for virology research. Nucleic Acids Res. 2012;40:D593–8. PubMed https://doi.org/10.1093/nar/gkr859

2. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. PubMed https://doi.org/10.1093/molbev/mst010

3. Price MN, Dehal PS, Arkin AP. FastTree 2—approximately maximum-likelihood trees for large alignments. PLoS One. 2010;5:e9490. PubMed https://doi.org/10.1371/journal.pone.0009490

4. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. PubMed https://doi.org/10.1093/bioinformatics/btu033

5. Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 2018;4:vey016. PubMed https://doi.org/10.1093/ve/vey016

6. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst Biol. 2018;67:901–4. PubMed https://doi.org/10.1093/sysbio/syy032

Appendix Table. Samples subjected to testing and the PCR and serology results

			Serology		
NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Ag NS1	IgM	IgG
BD-01	Bobo Dioulasso (BD)	Undetected			
BD-02	Bobo Dioulasso (BD)	Undetected			
BD-03	Bobo Dioulasso (BD)	Undetected			
BD-04	Bobo Dioulasso (BD)	Undetected			
BD-05	Bobo Dioulasso (BD)	Undetected			
BD-06	Bobo Dioulasso (BD)	Undetected			
BD-07	Bobo Dioulasso (BD)	Undetected			
BD-08	Bobo Dioulasso (BD)	Undetected			
BD-09	Bobo Dioulasso (BD)	Undetected			
BD-10	Bobo Dioulasso (BD)	Undetected			
BD-11	Bobo Dioulasso (BD)	Undetected			
BD-12	Bobo Dioulasso (BD)	Undetected			
BD-13	Bobo Dioulasso (BD)	Undetected			
BD-14	Bobo Dioulasso (BD)	Undetected			
BD-15	Bobo Dioulasso (BD)	Undetected			
BD-16	Bobo Dioulasso (BD)	Undetected			
BD-17	Bobo Dioulasso (BD)	Undetected			
BD-18	Bobo Dioulasso (BD)	Undetected			
BD-19	Bobo Dioulasso (BD)	Undetected			
BD-20	Bobo Dioulasso (BD)	Undetected			
BD-21	Bobo Dioulasso (BD)	Undetected			
BD-22	Bobo Dioulasso (BD)	Undetected			
BD-23	Bobo Dioulasso (BD)	Undetected			
BD-24	Bobo Dioulasso (BD)	Undetected			
BD-25	Bobo Dioulasso (BD)	Undetected			
BD-26	Bobo Dioulasso (BD)	Undetected			
BD-27	Bobo Dioulasso (BD)	Undetected			
BD-28	Bobo Dioulasso (BD)	Undetected			
BD-29	Bobo Dioulasso (BD)	Undetected			
BD-30	Bobo Dioulasso (BD)	Undetected			
BD-31	Bobo Dioulasso (BD)	Undetected			
BD-32	Bobo Dioulasso (BD)	Undetected			
BD-33	Bobo Dioulasso (BD)	Undetected			
BD-34	Bobo Dioulasso (BD)	Undetected			
BD-35	Bobo Dioulasso (BD)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
BD-36	Bobo Dioulasso (BD)	Undetected			
BD-37	Bobo Dioulasso (BD)	Undetected			
BD-38	Bobo Dioulasso (BD)	Undetected			
BD-39	Bobo Dioulasso (BD)	Undetected			
BD-40	Bobo Dioulasso (BD)	Undetected			
BD-41	Bobo Dioulasso (BD)	Undetected			
BD-42	Bobo Dioulasso (BD)	Undetected			
BD-43	Bobo Dioulasso (BD)	Undetected			
BD-44	Bobo Dioulasso (BD)	Undetected			
BD-45	Bobo Dioulasso (BD)	Undetected			
BD-46	Bobo Dioulasso (BD)	Undetected			
BD-47	Bobo Dioulasso (BD)	Undetected			
BD-48	Bobo Dioulasso (BD)	Undetected			
BD-49	Bobo Dioulasso (BD)	Undetected			
BD-50	Bobo Dioulasso (BD)	Undetected			
BD-51	Bobo Dioulasso (BD)	Undetected			
BD-52	Bobo Dioulasso (BD)	Undetected			
BD-53	Bobo Dioulasso (BD)	Undetected			
BD-54	Bobo Dioulasso (BD)	Undetected			
BD-55	Bobo Dioulasso (BD)	Undetected			
BD-56	Bobo Dioulasso (BD)	Undetected			
BD-57	Bobo Dioulasso (BD)	Undetected			
BD-58	Bobo Dioulasso (BD)	Undetected			
BD-59	Bobo Dioulasso (BD)	Undetected			
BD-60	Bobo Dioulasso (BD)	Undetected			
BD-61	Bobo Dioulasso (BD)	Undetected			
BD-62	Bobo Dioulasso (BD)	Undetected			
BD-63	Bobo Dioulasso (BD)	Undetected			
BD-64	Bobo Dioulasso (BD)	Undetected			
BD-65	Bobo Dioulasso (BD)	Undetected			
BD-66	Bobo Dioulasso (BD)	Undetected			
BD-67	Bobo Dioulasso (BD)	Undetected			
BD-68	Bobo Dioulasso (BD)	Undetected			
BD-69	Bobo Dioulasso (BD)	Undetected			
BD-70	Bobo Dioulasso (BD)	Undetected			
OS-01	Ouoga Secteur (OS)	Undetected			
OS-02	Ouoga Secteur (OS)	Undetected			
OS-03	Ouoga Secteur (OS)	Undetected			
OS-04	Ouoga Secteur (OS)	Undetected			
OS-05	Ouoga Secteur (OS)	Undetected			
OS-06	Ouoga Secteur (OS)	Undetected			
OS-07	Ouoga Secteur (OS)	Undetected			
OS-08	Ouoga Secteur (OS)	Undetected			
OS-09	Ouoga Secteur (OS)	Undetected			
OS-10	Ouoga Secteur (OS)	Undetected			
OS-11	Ouoga Secteur (OS)	Undetected			
OS-12	Ouoga Secteur (OS)	Undetected			
OS-13	Ouoga Secteur (OS)	Undetected			
OS-14	Ouoga Secteur (OS)	Undetected			
OS-15	Ouoga Secteur (OS)	Undetected			
OS-16	Ouoga Secteur (OS)	Undetected			
OS-17	Ouoga Secteur (OS)	Undetected			
OS-18	Ouoga Secteur (OS)	Undetected			
IP-001	Institute Pasteur (IP)	Undetected			
IP-002	Institute Pasteur (IP)	Undetected	Positive		
IP-003	Institute Pasteur (IP)	Undetected			
IP-004	Institute Pasteur (IP)	Undetected			
IP-005	Institute Pasteur (IP)	Undetected			
IP-006	Institute Pasteur (IP)	Undetected			
IP-007	Institute Pasteur (IP)	Undetected			
IP-008	Institute Pasteur (IP)	35.5	Positive		
IP-009	Institute Pasteur (IP)	37.1	Positive		
IP-010	Institute Pasteur (IP)	Undetected	Positive		
IP-011	Institute Pasteur (IP)	Undetected	Positive		
IP-012	Institute Pasteur (IP)	40.6	Positive		
IP-013	Institute Pasteur (IP)	Undetected			
IP-014	Institute Pasteur (IP)	Undetected			
IP-015	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP-016	Institute Pasteur (IP)	Undetected			
IP-017	Institute Pasteur (IP)	Undetected			
IP-018	Institute Pasteur (IP)	Undetected	Positive		
IP-019	Institute Pasteur (IP)	Undetected	Positive	Positive	Positive
IP-020	Institute Pasteur (IP)	Undetected	Positive		
IP-021	Institute Pasteur (IP)	Undetected			
IP-022	Institute Pasteur (IP)	Undetected			
IP-023	Institute Pasteur (IP)	Undetected			
IP-024	Institute Pasteur (IP)	Undetected			
IP-025	Institute Pasteur (IP)	Undetected			
IP-026	Institute Pasteur (IP)	Undetected			
IP-027	Institute Pasteur (IP)	Undetected			
IP-028	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-029	Institute Pasteur (IP)	37.5	Positive		
IP-030	Institute Pasteur (IP)	Undetected			
IP-031	Institute Pasteur (IP)	Undetected	Positive	Positive	
IP-032	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-033	Institute Pasteur (IP)	Undetected			
IP-034	Institute Pasteur (IP)	Undetected	Positive		Positive
IP-035	Institute Pasteur (IP)	Undetected			
IP-036	Institute Pasteur (IP)	40.6	Positive		
IP-037	Institute Pasteur (IP)	Undetected			
IP-038	Institute Pasteur (IP)	Undetected			
IP-039	Institute Pasteur (IP)	Undetected	Positive		
IP-040	Institute Pasteur (IP)	Undetected			
IP-041	Institute Pasteur (IP)	Undetected			
IP-042	Institute Pasteur (IP)	Undetected			
IP-043	Institute Pasteur (IP)	Undetected			
IP-044	Institute Pasteur (IP)	Undetected			
IP-045	Institute Pasteur (IP)	Undetected			
IP-046	Institute Pasteur (IP)	Undetected	Positive		
IP-047	Institute Pasteur (IP)	Undetected			
IP-048	Institute Pasteur (IP)	Undetected			
IP-049	Institute Pasteur (IP)	Undetected			
IP-050	Institute Pasteur (IP)	Undetected			
IP-051	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-052	Institute Pasteur (IP)	Undetected			
IP-053	Institute Pasteur (IP)	Undetected			
IP-054	Institute Pasteur (IP)	Undetected			
IP-055	Institute Pasteur (IP)	Undetected			
IP-056	Institute Pasteur (IP)	Undetected			
IP-057	Institute Pasteur (IP)	Undetected			
IP-058	Institute Pasteur (IP)	Undetected			
IP-059	Institute Pasteur (IP)	Undetected			
IP-060	Institute Pasteur (IP)	38.7			
IP-061	Institute Pasteur (IP)	Undetected			
IP-062	Institute Pasteur (IP)	Undetected			
IP-063	Institute Pasteur (IP)	Undetected			
IP-064	Institute Pasteur (IP)	Undetected			
IP-065	Institute Pasteur (IP)	Undetected			
IP-066	Institute Pasteur (IP)	Undetected			
IP-067	Institute Pasteur (IP)	Undetected			
IP-068	Institute Pasteur (IP)	Undetected			
IP-069	Institute Pasteur (IP)	Undetected			
IP-070	Institute Pasteur (IP)	Undetected			
IP-071	Institute Pasteur (IP)	Undetected			
IP-072	Institute Pasteur (IP)	Undetected			
IP-073	Institute Pasteur (IP)	Undetected			
IP-074	Institute Pasteur (IP)	Undetected			
IP-075	Institute Pasteur (IP)	Undetected			
IP-076	Institute Pasteur (IP)	Undetected			
IP-077	Institute Pasteur (IP)	Undetected			
IP-078	Institute Pasteur (IP)	Undetected			
IP-079	Institute Pasteur (IP)	Undetected			
IP-080	Institute Pasteur (IP)	Undetected			
IP-081	Institute Pasteur (IP)	Undetected			
IP-082	Institute Pasteur (IP)	Undetected			
IP-083	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP-084	Institute Pasteur (IP)	Undetected			
IP-085	Institute Pasteur (IP)	Undetected			
IP-086	Institute Pasteur (IP)	Undetected			
IP-087	Institute Pasteur (IP)	Undetected			
IP-088	Institute Pasteur (IP)	Undetected			
IP-089	Institute Pasteur (IP)	Undetected			
IP-090	Institute Pasteur (IP)	Undetected			
IP-091	Institute Pasteur (IP)	36.0	Positive		
IP-092	Institute Pasteur (IP)	Undetected	Positive	Positive	Positive
IP-093	Institute Pasteur (IP)	Undetected			
IP-094	Institute Pasteur (IP)	Undetected			
IP-095	Institute Pasteur (IP)	Undetected			
IP-096	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-097	Institute Pasteur (IP)	Undetected			
IP-098	Institute Pasteur (IP)	Undetected			
IP-099	Institute Pasteur (IP)	40.3	Positive		
IP-100	Institute Pasteur (IP)	Undetected			
IP-101	Institute Pasteur (IP)	Undetected			
IP-102	Institute Pasteur (IP)	Undetected			
IP-103	Institute Pasteur (IP)	34.6	Positive		
IP-104	Institute Pasteur (IP)	Undetected			
IP-105	Institute Pasteur (IP)	42.3	Positive		
IP-106	Institute Pasteur (IP)	Undetected			
IP-107	Institute Pasteur (IP)	Undetected			
IP-108	Institute Pasteur (IP)	Undetected			
IP-109	Institute Pasteur (IP)	Undetected			
IP-110	Institute Pasteur (IP)	Undetected			
IP-111	Institute Pasteur (IP)	Undetected			
IP-112	Institute Pasteur (IP)	39.5	Positive		
IP-113	Institute Pasteur (IP)	Undetected			
IP-114	Institute Pasteur (IP)	Undetected			
IP-115	Institute Pasteur (IP)	Undetected			
IP-116	Institute Pasteur (IP)	Undetected			
IP-117	Institute Pasteur (IP)	Undetected			
IP-118	Institute Pasteur (IP)	Undetected			
IP-119	Institute Pasteur (IP)	Undetected			
IP-120	Institute Pasteur (IP)	42.2	Positive		
IP-121	Institute Pasteur (IP)	29.0	Positive		
IP-122	Institute Pasteur (IP)	Undetected			
IP-123	Institute Pasteur (IP)	Undetected			
IP-124	Institute Pasteur (IP)	Undetected	Positive	Positive	
IP-125	Institute Pasteur (IP)	Undetected			
IP-126	Institute Pasteur (IP)	Undetected			
IP-127	Institute Pasteur (IP)	38.2			
IP-128	Institute Pasteur (IP)	Undetected			
IP-129	Institute Pasteur (IP)	Undetected			
IP-130	Institute Pasteur (IP)	Undetected			
IP-131	Institute Pasteur (IP)	Undetected			
IP-132	Institute Pasteur (IP)	Undetected			
IP-133	Institute Pasteur (IP)	Undetected			
IP-134	Institute Pasteur (IP)	Undetected			
IP-135	Institute Pasteur (IP)	Undetected			
IP-136	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-137	Institute Pasteur (IP)	Undetected			
IP-138	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-139	Institute Pasteur (IP)	Undetected			
IP-140	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-141	Institute Pasteur (IP)	Undetected			
IP-142	Institute Pasteur (IP)	Undetected			
IP-143	Institute Pasteur (IP)	Undetected			
IP-144	Institute Pasteur (IP)	Undetected			
IP-145	Institute Pasteur (IP)	Undetected			
IP-146	Institute Pasteur (IP)	Undetected	Positive		
IP-147	Institute Pasteur (IP)	Undetected			
IP-148	Institute Pasteur (IP)	Undetected			
IP-149	Institute Pasteur (IP)	Undetected			
IP-150	Institute Pasteur (IP)	Undetected			
IP-151	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP-152	Institute Pasteur (IP)	Undetected			
IP-153	Institute Pasteur (IP)	33.7	Positive		
IP-154	Institute Pasteur (IP)	39.9	Positive		
IP-155	Institute Pasteur (IP)	Undetected			
IP-156	Institute Pasteur (IP)	Undetected			
IP-157	Institute Pasteur (IP)	Undetected			
IP-158	Institute Pasteur (IP)	Undetected			
IP-159	Institute Pasteur (IP)	32.0	Positive		
IP-160	Institute Pasteur (IP)	Undetected			
IP-161	Institute Pasteur (IP)	Undetected			
IP-162	Institute Pasteur (IP)	Undetected			
IP-163	Institute Pasteur (IP)	Undetected			
IP-164	Institute Pasteur (IP)	Undetected			
IP-165	Institute Pasteur (IP)	Undetected		Positive	Positive
IP-166	Institute Pasteur (IP)	Undetected			
IP-167	Institute Pasteur (IP)	Undetected			
IP-168	Institute Pasteur (IP)	Undetected			
IP-169	Institute Pasteur (IP)	Undetected			
IP-170	Institute Pasteur (IP)	Undetected	Positive	Positive	Positive
IP-171	Institute Pasteur (IP)	33.8	Positive	Positive	Positive
IP-172	Institute Pasteur (IP)	Undetected			
IP-173	Institute Pasteur (IP)	Undetected			
IP-174	Institute Pasteur (IP)	Undetected			
IP-175	Institute Pasteur (IP)	Undetected			
IP-176	Institute Pasteur (IP)	Undetected			
IP-177	Institute Pasteur (IP)	Undetected			
IP-178	Institute Pasteur (IP)	Undetected			
IP-179	Institute Pasteur (IP)	22.5	Positive		
IP-180	Institute Pasteur (IP)	Undetected			
IP-181	Institute Pasteur (IP)	Undetected			
IP-182	Institute Pasteur (IP)	Undetected			
IP-183	Institute Pasteur (IP)	Undetected			
IP-184	Institute Pasteur (IP)	Undetected			
IP-185	Institute Pasteur (IP)	Undetected			
IP-186	Institute Pasteur (IP)	Undetected			
IP-187	Institute Pasteur (IP)	Undetected			
IP-188	Institute Pasteur (IP)	Undetected			
IP-189	Institute Pasteur (IP)	Undetected			
IP-190	Institute Pasteur (IP)	Undetected			
IP-191	Institute Pasteur (IP)	Undetected			
IP-192	Institute Pasteur (IP)	Undetected			
IP-193	Institute Pasteur (IP)	Undetected			
IP-194	Institute Pasteur (IP)	25.2			
IP-195	Institute Pasteur (IP)	41.6			
IP-196	Institute Pasteur (IP)	Undetected			
IP-197	Institute Pasteur (IP)	Undetected			
IP-198	Institute Pasteur (IP)	Undetected			
IP-199	Institute Pasteur (IP)	Undetected			
IP-200	Institute Pasteur (IP)	Undetected			
IP-201	Institute Pasteur (IP)	Undetected			
IP-202	Institute Pasteur (IP)	Undetected			
IP-203	Institute Pasteur (IP)	Undetected			
IP-204	Institute Pasteur (IP)	Undetected			
IP-205	Institute Pasteur (IP)	Undetected			
IP-206	Institute Pasteur (IP)	Undetected			
IP-207	Institute Pasteur (IP)	Undetected			
IP-208	Institute Pasteur (IP)	Undetected			
IP-209	Institute Pasteur (IP)	Undetected			
IP-210	Institute Pasteur (IP)	Undetected			
IP-211	Institute Pasteur (IP)	Undetected			
IP-212	Institute Pasteur (IP)	Undetected			
IP-213	Institute Pasteur (IP)	Undetected			
IP-214	Institute Pasteur (IP)	Undetected			
IP-215	Institute Pasteur (IP)	Undetected			
IP-216	Institute Pasteur (IP)	Undetected			
IP-217	Institute Pasteur (IP)	Undetected			
IP-218	Institute Pasteur (IP)	Undetected			
IP-219	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP-220	Institute Pasteur (IP)	Undetected			
IP-221	Institute Pasteur (IP)	Undetected			
IP-222	Institute Pasteur (IP)	Undetected			
IP-223	Institute Pasteur (IP)	Undetected			
IP-224	Institute Pasteur (IP)	Undetected			
IP-225	Institute Pasteur (IP)	Undetected			
IP-226	Institute Pasteur (IP)	25.4			
IP-227	Institute Pasteur (IP)	Undetected			
IP-228	Institute Pasteur (IP)	Undetected			
IP-229	Institute Pasteur (IP)	Undetected			
IP-230	Institute Pasteur (IP)	Undetected			
IP-231	Institute Pasteur (IP)	Undetected			
IP-232	Institute Pasteur (IP)	Undetected			
IP-233	Institute Pasteur (IP)	Undetected			
IP-234	Institute Pasteur (IP)	Undetected			
IP-235	Institute Pasteur (IP)	Undetected			
IP-236	Institute Pasteur (IP)	Undetected			
IP-237	Institute Pasteur (IP)	Undetected			
IP-238	Institute Pasteur (IP)	Undetected			
IP-239	Institute Pasteur (IP)	Undetected			
IP-240	Institute Pasteur (IP)	Undetected			
IP-241	Institute Pasteur (IP)	Undetected			
IP-242	Institute Pasteur (IP)	29.5			
IP-243	Institute Pasteur (IP)	Undetected			
IP-244	Institute Pasteur (IP)	Undetected			
IP-245	Institute Pasteur (IP)	Undetected			
IP-246	Institute Pasteur (IP)	31.1			
IP-247	Institute Pasteur (IP)	Undetected			
IP-248	Institute Pasteur (IP)	Undetected			
IP-249	Institute Pasteur (IP)	Undetected			
IP-250	Institute Pasteur (IP)	Undetected			
IP-251	Institute Pasteur (IP)	Undetected			
IP-252	Institute Pasteur (IP)	Undetected			
IP-253	Institute Pasteur (IP)	Undetected			
IP-254	Institute Pasteur (IP)	Undetected			
IP-255	Institute Pasteur (IP)	Undetected			
IP-256	Institute Pasteur (IP)	Undetected			
IP-257	Institute Pasteur (IP)	Undetected			
IP-258	Institute Pasteur (IP)	Undetected			
IP-259	Institute Pasteur (IP)	Undetected			
IP-260	Institute Pasteur (IP)	Undetected			
IP-261	Institute Pasteur (IP)	Undetected			
IP-262	Institute Pasteur (IP)	Undetected			
IP-263	Institute Pasteur (IP)	Undetected			
IP-264	Institute Pasteur (IP)	Undetected			
IP-265	Institute Pasteur (IP)	Undetected			
IP-266	Institute Pasteur (IP)	Undetected			
IP-267	Institute Pasteur (IP)	19.3			
IP-268	Institute Pasteur (IP)	Undetected			
IP-269	Institute Pasteur (IP)	Undetected			
IP-270	Institute Pasteur (IP)	37.1			
IP-271	Institute Pasteur (IP)	Undetected			
IP-272	Institute Pasteur (IP)	Undetected			
IP-273	Institute Pasteur (IP)	Undetected			
IP-274	Institute Pasteur (IP)	Undetected			
IP-275	Institute Pasteur (IP)	Undetected			
IP-276	Institute Pasteur (IP)	Undetected			
IP-277	Institute Pasteur (IP)	Undetected			
IP-278	Institute Pasteur (IP)	Undetected			
IP-279	Institute Pasteur (IP)	Undetected			
IP-280	Institute Pasteur (IP)	Undetected			
IP-281	Institute Pasteur (IP)	Undetected			
IP-282	Institute Pasteur (IP)	Undetected			
IP-283	Institute Pasteur (IP)	Undetected			
IP-284	Institute Pasteur (IP)	Undetected			
IP-285	Institute Pasteur (IP)	Undetected			
IP-286	Institute Pasteur (IP)	Undetected			
IP-287	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP-288	Institute Pasteur (IP)	Undetected			
IP-289	Institute Pasteur (IP)	Undetected			
IP-290	Institute Pasteur (IP)	Undetected			
IP-291	Institute Pasteur (IP)	Undetected			
IP-292	Institute Pasteur (IP)	Undetected			
IP-293	Institute Pasteur (IP)	Undetected			
IP-294	Institute Pasteur (IP)	Undetected			
IP-295	Institute Pasteur (IP)	Undetected			
IP-296	Institute Pasteur (IP)	Undetected			
IP-297	Institute Pasteur (IP)	Undetected			
IP-298	Institute Pasteur (IP)	Undetected	Positive		
IP-299	Institute Pasteur (IP)	Undetected			
IP-300	Institute Pasteur (IP)	Undetected			
IP-301	Institute Pasteur (IP)	Undetected			
IP-302	Institute Pasteur (IP)	Undetected	Positive	Positive	Positive
IP-303	Institute Pasteur (IP)	Undetected			
IP-304	Institute Pasteur (IP)	24.1	Positive		
IP-305	Institute Pasteur (IP)	Undetected			
IP-306	Institute Pasteur (IP)	Undetected			
IP-307	Institute Pasteur (IP)	37.3			
IP-308	Institute Pasteur (IP)	Undetected			
IP-309	Institute Pasteur (IP)	Undetected			
IP-310	Institute Pasteur (IP)	30.3	Positive		Positive
IP-311	Institute Pasteur (IP)	Undetected			
IP-312	Institute Pasteur (IP)	Undetected			
IP-313	Institute Pasteur (IP)	Undetected			
IP-314	Institute Pasteur (IP)	23.8	Positive		
IP-315	Institute Pasteur (IP)	Undetected			
IP-316	Institute Pasteur (IP)	Undetected			
IP-317	Institute Pasteur (IP)	Undetected			
IP-318	Institute Pasteur (IP)	Undetected			
IP-319	Institute Pasteur (IP)	Undetected			
IP-320	Institute Pasteur (IP)	Undetected			
IP-321	Institute Pasteur (IP)	Undetected			
IP-322	Institute Pasteur (IP)	Undetected			
IP-323	Institute Pasteur (IP)	Undetected			
IP-324	Institute Pasteur (IP)	Undetected			
IP 325	Institute Pasteur (IP)	Undetected			
IP 326	Institute Pasteur (IP)	Undetected			Positive
IP 327	Institute Pasteur (IP)	Undetected			
IP 328	Institute Pasteur (IP)	Undetected			
IP 329	Institute Pasteur (IP)	Undetected			Positive
IP 330	Institute Pasteur (IP)	Undetected			
IP 331	Institute Pasteur (IP)	Undetected			
IP 332	Institute Pasteur (IP)	Undetected			
IP 333	Institute Pasteur (IP)	Undetected			
IP 334	Institute Pasteur (IP)	Undetected			
IP 335	Institute Pasteur (IP)	Undetected			
IP 336	Institute Pasteur (IP)	Undetected			
IP 337	Institute Pasteur (IP)	Undetected			
IP 338	Institute Pasteur (IP)	Undetected			
IP 339	Institute Pasteur (IP)	Undetected			
IP 340	Institute Pasteur (IP)	Undetected			
IP 341	Institute Pasteur (IP)	Undetected			
IP 342	Institute Pasteur (IP)	Undetected			
IP 343	Institute Pasteur (IP)	Undetected			
IP 344	Institute Pasteur (IP)	Undetected			Positive
IP 345	Institute Pasteur (IP)	Undetected			
IP 346	Institute Pasteur (IP)	Undetected			
IP 347	Institute Pasteur (IP)	Undetected			
IP 348	Institute Pasteur (IP)	Undetected			
IP 349	Institute Pasteur (IP)	Undetected			
IP 350	Institute Pasteur (IP)	Undetected			
IP 351	Institute Pasteur (IP)	Undetected			Positive
IP 352	Institute Pasteur (IP)	Undetected			
IP 353	Institute Pasteur (IP)	Undetected			Positive
IP 354	Institute Pasteur (IP)	Undetected			
IP 355	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 356	Institute Pasteur (IP)	Undetected			Positive
IP 357	Institute Pasteur (IP)	Undetected			
IP 358	Institute Pasteur (IP)	Undetected			Positive
IP 359	Institute Pasteur (IP)	Undetected			
IP 360	Institute Pasteur (IP)	Undetected			
IP 361	Institute Pasteur (IP)	Undetected			
IP 362	Institute Pasteur (IP)	Undetected			Positive
IP 363	Institute Pasteur (IP)	Undetected			
IP 364	Institute Pasteur (IP)	Undetected			
IP 365	Institute Pasteur (IP)	Undetected			
IP 366	Institute Pasteur (IP)	Undetected			
IP 367	Institute Pasteur (IP)	Undetected			
IP 368	Institute Pasteur (IP)	Undetected			Positive
IP 369	Institute Pasteur (IP)	Undetected		Positive	Positive
IP 370	Institute Pasteur (IP)	Undetected			
IP 371	Institute Pasteur (IP)	Undetected			
IP 372	Institute Pasteur (IP)	Undetected			
IP 373	Institute Pasteur (IP)	Undetected			
IP 374	Institute Pasteur (IP)	Undetected			Positive
IP 375	Institute Pasteur (IP)	Undetected			
IP 376	Institute Pasteur (IP)	Undetected			
IP 377	Institute Pasteur (IP)	Undetected			
IP 378	Institute Pasteur (IP)	Undetected			
IP 379	Institute Pasteur (IP)	Undetected			
IP 380	Institute Pasteur (IP)	Undetected			
IP 381	Institute Pasteur (IP)	Undetected			
IP 382	Institute Pasteur (IP)	Undetected			
IP 383	Institute Pasteur (IP)	Undetected			
IP 384	Institute Pasteur (IP)	Undetected			
IP 385	Institute Pasteur (IP)	Undetected			
IP 386	Institute Pasteur (IP)	Undetected			
IP 387	Institute Pasteur (IP)	Undetected	Positive		
IP 388	Institute Pasteur (IP)	Undetected			
IP 389	Institute Pasteur (IP)	Undetected			
IP 390	Institute Pasteur (IP)	Undetected			
IP 391	Institute Pasteur (IP)	Undetected			
IP 392	Institute Pasteur (IP)	Undetected			
IP 393	Institute Pasteur (IP)	Undetected			
IP 394	Institute Pasteur (IP)	Undetected			
IP 395	Institute Pasteur (IP)	Undetected			
IP 396	Institute Pasteur (IP)	Undetected			
IP 397	Institute Pasteur (IP)	Undetected			
IP 398	Institute Pasteur (IP)	Undetected			
IP 399	Institute Pasteur (IP)	Undetected			
IP 400	Institute Pasteur (IP)	Undetected			
IP 401	Institute Pasteur (IP)	Undetected			
IP 402	Institute Pasteur (IP)	Undetected			
IP 403	Institute Pasteur (IP)	Undetected			
IP 404	Institute Pasteur (IP)	Undetected			
IP 405	Institute Pasteur (IP)	Undetected			
IP 406	Institute Pasteur (IP)	Undetected			
IP 407	Institute Pasteur (IP)	Undetected			
IP 408	Institute Pasteur (IP)	Undetected			
IP 409	Institute Pasteur (IP)	Undetected			
IP 410	Institute Pasteur (IP)	Undetected			
IP 411	Institute Pasteur (IP)	Undetected			
IP 412	Institute Pasteur (IP)	Undetected			
IP 413	Institute Pasteur (IP)	Undetected			
IP 414	Institute Pasteur (IP)	Undetected			
IP 415	Institute Pasteur (IP)	Undetected			
IP 416	Institute Pasteur (IP)	Undetected			
IP 417	Institute Pasteur (IP)	Undetected			
IP 418	Institute Pasteur (IP)	Undetected			
IP 419	Institute Pasteur (IP)	Undetected			
IP 420	Institute Pasteur (IP)	Undetected			
IP 421	Institute Pasteur (IP)	Undetected			
IP 422	Institute Pasteur (IP)	Undetected			
IP 423	Institute Pasteur (IP)	Undetected			

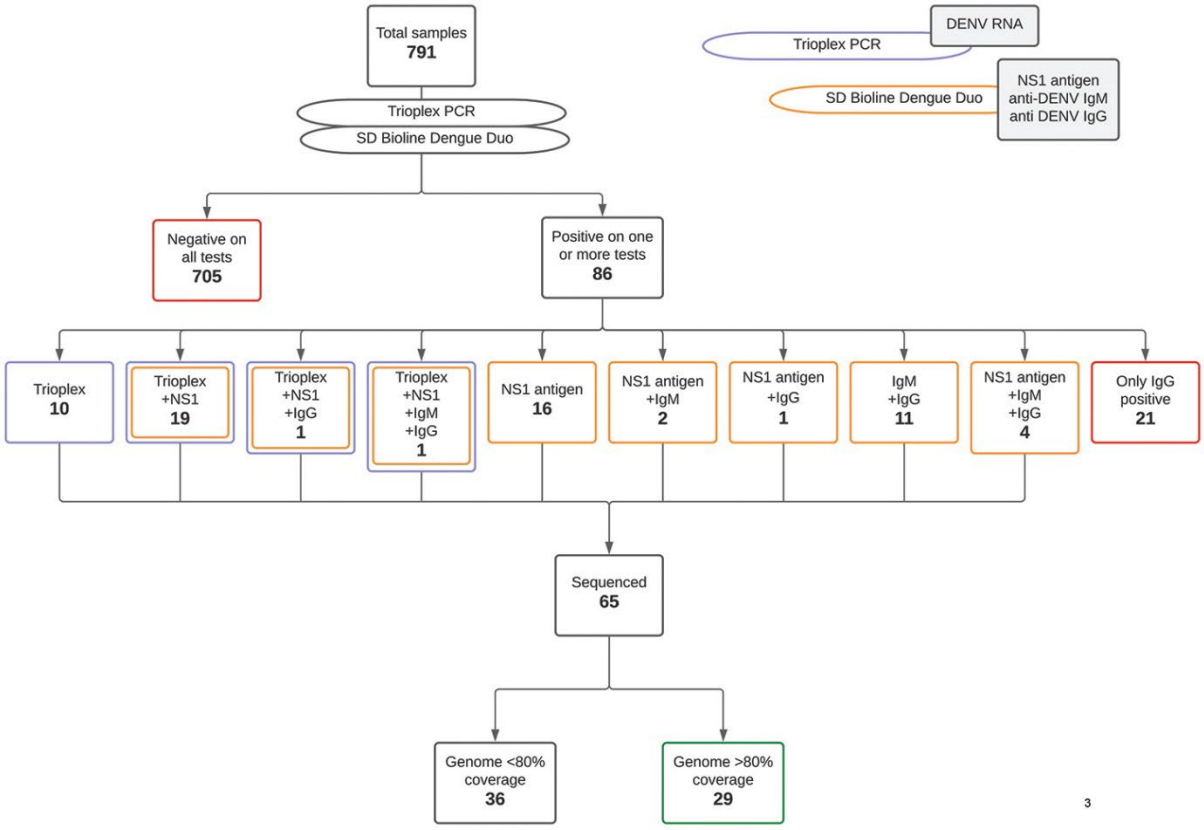
NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 424	Institute Pasteur (IP)	Undetected			
IP 425	Institute Pasteur (IP)	Undetected			
IP 426	Institute Pasteur (IP)	Undetected			
IP 427	Institute Pasteur (IP)	Undetected			
IP 428	Institute Pasteur (IP)	Undetected			
IP 429	Institute Pasteur (IP)	Undetected			
IP 430	Institute Pasteur (IP)	Undetected			
IP 431	Institute Pasteur (IP)	Undetected			
IP 432	Institute Pasteur (IP)	Undetected			
IP 433	Institute Pasteur (IP)	Undetected			
IP 434	Institute Pasteur (IP)	Undetected			
IP 435	Institute Pasteur (IP)	Undetected			
IP 436	Institute Pasteur (IP)	Undetected			
IP 437	Institute Pasteur (IP)	Undetected			
IP 438	Institute Pasteur (IP)	Undetected			
IP 439	Institute Pasteur (IP)	Undetected			
IP 440	Institute Pasteur (IP)	Undetected			
IP 441	Institute Pasteur (IP)	Undetected			
IP 442	Institute Pasteur (IP)	Undetected			
IP 443	Institute Pasteur (IP)	Undetected			
IP 444	Institute Pasteur (IP)	Undetected			
IP 445	Institute Pasteur (IP)	Undetected			
IP 446	Institute Pasteur (IP)	Undetected			
IP 447	Institute Pasteur (IP)	Undetected			
IP 448	Institute Pasteur (IP)	Undetected			
IP 449	Institute Pasteur (IP)	Undetected			
IP 450	Institute Pasteur (IP)	Undetected			Positive
IP 451	Institute Pasteur (IP)	Undetected			
IP 452	Institute Pasteur (IP)	Undetected			
IP 453	Institute Pasteur (IP)	Undetected			Positive
IP 454	Institute Pasteur (IP)	Undetected			
IP 455	Institute Pasteur (IP)	Undetected			
IP 456	Institute Pasteur (IP)	Undetected			
IP 457	Institute Pasteur (IP)	Undetected			
IP 458	Institute Pasteur (IP)	Undetected			
IP 459	Institute Pasteur (IP)	Undetected			
IP 460	Institute Pasteur (IP)	Undetected			
IP 461	Institute Pasteur (IP)	Undetected			
IP 462	Institute Pasteur (IP)	Undetected			
IP 463	Institute Pasteur (IP)	Undetected			
IP 464	Institute Pasteur (IP)	Undetected			Positive
IP 465	Institute Pasteur (IP)	Undetected			
IP 466	Institute Pasteur (IP)	Undetected			
IP 467	Institute Pasteur (IP)	Undetected			
IP 468	Institute Pasteur (IP)	Undetected			
IP 469	Institute Pasteur (IP)	Undetected			
IP 470	Institute Pasteur (IP)	Undetected			
IP 471	Institute Pasteur (IP)	Undetected			
IP 472	Institute Pasteur (IP)	Undetected			
IP 473	Institute Pasteur (IP)	Undetected			
IP 474	Institute Pasteur (IP)	Undetected			
IP 475	Institute Pasteur (IP)	Undetected			
IP 476	Institute Pasteur (IP)	Undetected			Positive
IP 477	Institute Pasteur (IP)	Undetected			
IP 478	Institute Pasteur (IP)	Undetected			
IP 479	Institute Pasteur (IP)	Undetected			
IP 480	Institute Pasteur (IP)	Undetected			
IP 481	Institute Pasteur (IP)	Undetected			
IP 482	Institute Pasteur (IP)	Undetected			
IP 483	Institute Pasteur (IP)	Undetected			
IP 484	Institute Pasteur (IP)	Undetected			
IP 485	Institute Pasteur (IP)	Undetected			
IP 486	Institute Pasteur (IP)	Undetected			
IP 487	Institute Pasteur (IP)	Undetected			
IP 488	Institute Pasteur (IP)	Undetected			
IP 489	Institute Pasteur (IP)	Undetected			
IP 490	Institute Pasteur (IP)	Undetected			
IP 491	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 492	Institute Pasteur (IP)	Undetected			
IP 493	Institute Pasteur (IP)	Undetected			
IP 494	Institute Pasteur (IP)	41.2	Positive		
IP 495	Institute Pasteur (IP)	Undetected			
IP 496	Institute Pasteur (IP)	Undetected			
IP 497	Institute Pasteur (IP)	Undetected			
IP 498	Institute Pasteur (IP)	Undetected			
IP 499	Institute Pasteur (IP)	Undetected			
IP 500	Institute Pasteur (IP)	Undetected			
IP 501	Institute Pasteur (IP)	Undetected			
IP 502	Institute Pasteur (IP)	Undetected			
IP 503	Institute Pasteur (IP)	Undetected			
IP 504	Institute Pasteur (IP)	Undetected			
IP 505	Institute Pasteur (IP)	Undetected			
IP 506	Institute Pasteur (IP)	Undetected			
IP 507	Institute Pasteur (IP)	Undetected	Positive		
IP 508	Institute Pasteur (IP)	Undetected			
IP 509	Institute Pasteur (IP)	Undetected			
IP 510	Institute Pasteur (IP)	Undetected			
IP 511	Institute Pasteur (IP)	Undetected			
IP 512	Institute Pasteur (IP)	Undetected			
IP 513	Institute Pasteur (IP)	Undetected			
IP 514	Institute Pasteur (IP)	Undetected			
IP 515	Institute Pasteur (IP)	Undetected			
IP 516	Institute Pasteur (IP)	Undetected			
IP 517	Institute Pasteur (IP)	Undetected			
IP 518	Institute Pasteur (IP)	Undetected			
IP 519	Institute Pasteur (IP)	Undetected			
IP 520	Institute Pasteur (IP)	Undetected			
IP 521	Institute Pasteur (IP)	Undetected			
IP 522	Institute Pasteur (IP)	Undetected			
IP 523	Institute Pasteur (IP)	Undetected			
IP 524	Institute Pasteur (IP)	Undetected			
IP 525	Institute Pasteur (IP)	Undetected			
IP 526	Institute Pasteur (IP)	Undetected			
IP 527	Institute Pasteur (IP)	Undetected			
IP 528	Institute Pasteur (IP)	Undetected			
IP 529	Institute Pasteur (IP)	Undetected			
IP 530	Institute Pasteur (IP)	Undetected			
IP 531	Institute Pasteur (IP)	Undetected			
IP 532	Institute Pasteur (IP)	Undetected			
IP 533	Institute Pasteur (IP)	Undetected	Positive		
IP 534	Institute Pasteur (IP)	Undetected			
IP 535	Institute Pasteur (IP)	Undetected			
IP 536	Institute Pasteur (IP)	Undetected		Positive	Positive
IP 537	Institute Pasteur (IP)	Undetected			
IP 538	Institute Pasteur (IP)	Undetected	Positive		
IP 539	Institute Pasteur (IP)	Undetected			
IP 540	Institute Pasteur (IP)	Undetected	Positive		
IP 541	Institute Pasteur (IP)	Undetected			
IP 542	Institute Pasteur (IP)	Undetected			
IP 543	Institute Pasteur (IP)	Undetected			
IP 544	Institute Pasteur (IP)	Undetected			Positive
IP 545	Institute Pasteur (IP)	Undetected			
IP 546	Institute Pasteur (IP)	Undetected			
IP 547	Institute Pasteur (IP)	Undetected			
IP 548	Institute Pasteur (IP)	Undetected			
IP 549	Institute Pasteur (IP)	Undetected			
IP 550	Institute Pasteur (IP)	Undetected			
IP 551	Institute Pasteur (IP)	Undetected			
IP 552	Institute Pasteur (IP)	Undetected			
IP 553	Institute Pasteur (IP)	Undetected			
IP 554	Institute Pasteur (IP)	Undetected			
IP 555	Institute Pasteur (IP)	Undetected			
IP 556	Institute Pasteur (IP)	Undetected			
IP 557	Institute Pasteur (IP)	Undetected			
IP 558	Institute Pasteur (IP)	Undetected			
IP 559	Institute Pasteur (IP)	Undetected			

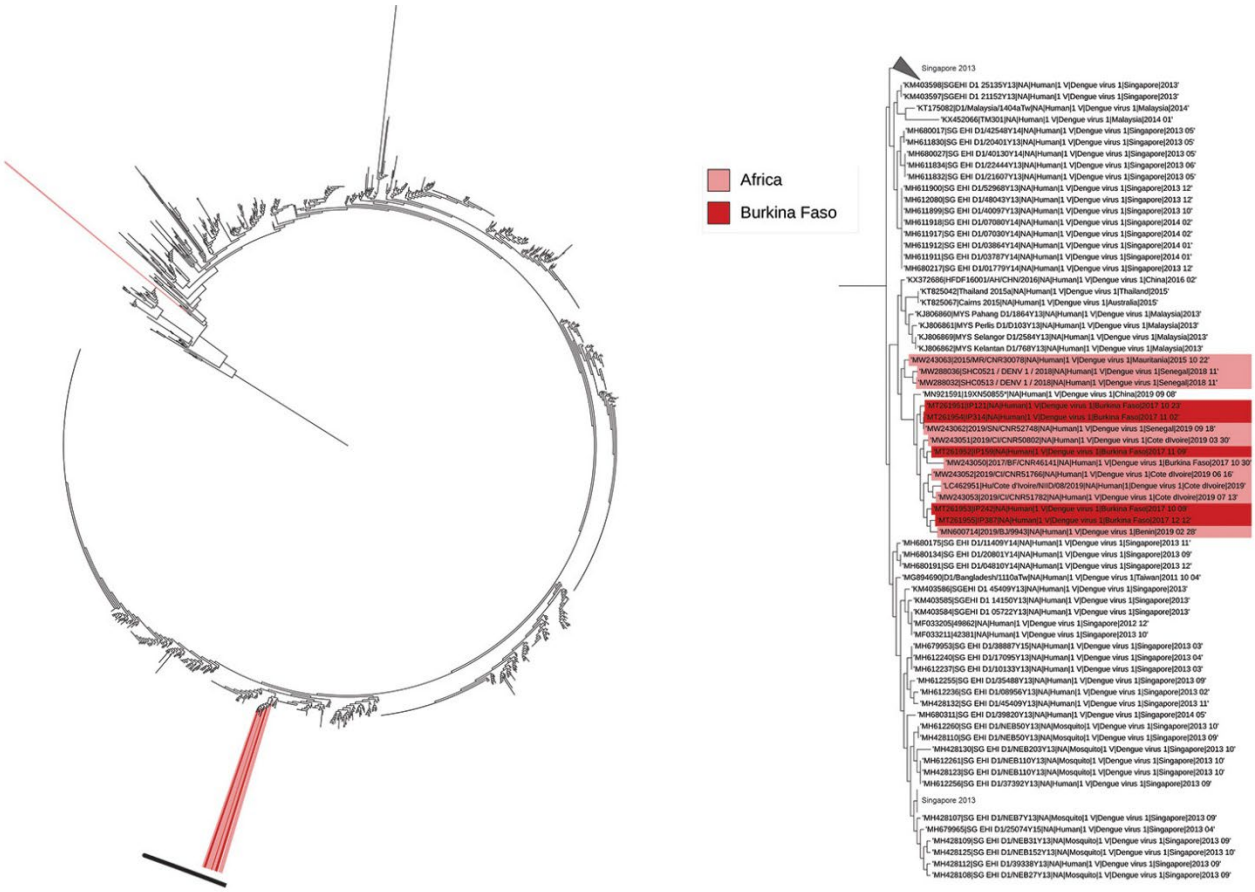
NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 560	Institute Pasteur (IP)	Undetected	Positive		
IP 561	Institute Pasteur (IP)	Undetected			
IP 562	Institute Pasteur (IP)	Undetected			
IP 563	Institute Pasteur (IP)	Undetected			
IP 564	Institute Pasteur (IP)	Undetected			
IP 565	Institute Pasteur (IP)	Undetected			
IP 566	Institute Pasteur (IP)	Undetected			
IP 567	Institute Pasteur (IP)	Undetected			
IP 568	Institute Pasteur (IP)	Undetected			
IP 569	Institute Pasteur (IP)	Undetected			
IP 570	Institute Pasteur (IP)	Undetected			
IP 571	Institute Pasteur (IP)	Undetected			
IP 572	Institute Pasteur (IP)	Undetected			
IP 573	Institute Pasteur (IP)	Undetected			
IP 574	Institute Pasteur (IP)	Undetected			
IP 575	Institute Pasteur (IP)	Undetected			
IP 576	Institute Pasteur (IP)	Undetected			
IP 577	Institute Pasteur (IP)	Undetected			
IP 578	Institute Pasteur (IP)	Undetected			Positive
IP 579	Institute Pasteur (IP)	Undetected			
IP 580	Institute Pasteur (IP)	Undetected			
IP 581	Institute Pasteur (IP)	Undetected			
IP 582	Institute Pasteur (IP)	Undetected			
IP 583	Institute Pasteur (IP)	Undetected			
IP 584	Institute Pasteur (IP)	Undetected			
IP 585	Institute Pasteur (IP)	Undetected			Positive
IP 586	Institute Pasteur (IP)	Undetected			
IP 587	Institute Pasteur (IP)	Undetected			
IP 588	Institute Pasteur (IP)	Undetected			
IP 589	Institute Pasteur (IP)	Undetected			
IP 590	Institute Pasteur (IP)	Undetected			
IP 591	Institute Pasteur (IP)	Undetected			
IP 592	Institute Pasteur (IP)	Undetected			
IP 593	Institute Pasteur (IP)	Undetected			
IP 594	Institute Pasteur (IP)	Undetected			
IP 595	Institute Pasteur (IP)	Undetected			
IP 596	Institute Pasteur (IP)	Undetected			
IP 597	Institute Pasteur (IP)	Undetected			
IP 598	Institute Pasteur (IP)	Undetected			
IP 599	Institute Pasteur (IP)	Undetected			
IP 600	Institute Pasteur (IP)	Undetected			
IP 601	Institute Pasteur (IP)	Undetected			
IP 602	Institute Pasteur (IP)	Undetected			
IP 603	Institute Pasteur (IP)	Undetected			
IP 604	Institute Pasteur (IP)	Undetected			
IP 605	Institute Pasteur (IP)	Undetected			
IP 606	Institute Pasteur (IP)	Undetected			
IP 607	Institute Pasteur (IP)	Undetected			
IP 608	Institute Pasteur (IP)	Undetected			
IP 609	Institute Pasteur (IP)	Undetected			
IP 610	Institute Pasteur (IP)	Undetected			
IP 611	Institute Pasteur (IP)	Undetected			
IP 612	Institute Pasteur (IP)	Undetected			
IP 613	Institute Pasteur (IP)	Undetected			
IP 614	Institute Pasteur (IP)	Undetected			
IP 615	Institute Pasteur (IP)	Undetected			
IP 616	Institute Pasteur (IP)	Undetected			
IP 617	Institute Pasteur (IP)	Undetected			
IP 618	Institute Pasteur (IP)	Undetected			
IP 619	Institute Pasteur (IP)	Undetected			
IP 620	Institute Pasteur (IP)	Undetected			Positive
IP 621	Institute Pasteur (IP)	Undetected			
IP 622	Institute Pasteur (IP)	Undetected			
IP 623	Institute Pasteur (IP)	Undetected			
IP 624	Institute Pasteur (IP)	Undetected			
IP 625	Institute Pasteur (IP)	Undetected			
IP 626	Institute Pasteur (IP)	Undetected			
IP 627	Institute Pasteur (IP)	Undetected			

NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 628	Institute Pasteur (IP)	Undetected			
IP 629	Institute Pasteur (IP)	Undetected			
IP 630	Institute Pasteur (IP)	Undetected			
IP 631	Institute Pasteur (IP)	Undetected			
IP 632	Institute Pasteur (IP)	Undetected			
IP 633	Institute Pasteur (IP)	Undetected			
IP 634	Institute Pasteur (IP)	Undetected			
IP 635	Institute Pasteur (IP)	Undetected			
IP 636	Institute Pasteur (IP)	Undetected			
IP 637	Institute Pasteur (IP)	Undetected			
IP 638	Institute Pasteur (IP)	Undetected			
IP 639	Institute Pasteur (IP)	Undetected			
IP 640	Institute Pasteur (IP)	Undetected			
IP 641	Institute Pasteur (IP)	Undetected		Positive	Positive
IP 642	Institute Pasteur (IP)	Undetected			
IP 643	Institute Pasteur (IP)	Undetected			
IP 644	Institute Pasteur (IP)	Undetected			
IP 645	Institute Pasteur (IP)	Undetected			
IP 646	Institute Pasteur (IP)	Undetected			
IP 647	Institute Pasteur (IP)	Undetected			
IP 648	Institute Pasteur (IP)	Undetected			
IP 649	Institute Pasteur (IP)	Undetected			
IP 650	Institute Pasteur (IP)	Undetected			
IP 651	Institute Pasteur (IP)	Undetected			
IP 652	Institute Pasteur (IP)	Undetected			
IP 653	Institute Pasteur (IP)	Undetected			
IP 654	Institute Pasteur (IP)	Undetected			
IP 655	Institute Pasteur (IP)	Undetected			
IP 656	Institute Pasteur (IP)	Undetected			
IP 657	Institute Pasteur (IP)	Undetected			
IP 658	Institute Pasteur (IP)	Undetected			
IP 659	Institute Pasteur (IP)	Undetected			
IP 660	Institute Pasteur (IP)	Undetected			
IP 661	Institute Pasteur (IP)	Undetected			
IP 662	Institute Pasteur (IP)	Undetected			
IP 663	Institute Pasteur (IP)	Undetected			
IP 664	Institute Pasteur (IP)	Undetected			
IP 665	Institute Pasteur (IP)	Undetected			
IP 666	Institute Pasteur (IP)	Undetected	Positive		
IP 667	Institute Pasteur (IP)	Undetected			
IP 668	Institute Pasteur (IP)	Undetected			
IP 669	Institute Pasteur (IP)	Undetected			Positive
IP 670	Institute Pasteur (IP)	Undetected			
IP 671	Institute Pasteur (IP)	Undetected			
IP 672	Institute Pasteur (IP)	Undetected			
IP 673	Institute Pasteur (IP)	Undetected			
IP 674	Institute Pasteur (IP)	Undetected			Positive
IP 675	Institute Pasteur (IP)	Undetected			
IP 676	Institute Pasteur (IP)	Undetected			
IP 677	Institute Pasteur (IP)	Undetected			Positive
IP 678	Institute Pasteur (IP)	Undetected			
IP 679	Institute Pasteur (IP)	Undetected			
IP 680	Institute Pasteur (IP)	Undetected			
IP 681	Institute Pasteur (IP)	Undetected			
IP 682	Institute Pasteur (IP)	Undetected			
IP 683	Institute Pasteur (IP)	Undetected			
IP 684	Institute Pasteur (IP)	Undetected			
IP 685	Institute Pasteur (IP)	Undetected			
IP 686	Institute Pasteur (IP)	Undetected			
IP 687	Institute Pasteur (IP)	Undetected			
IP 688	Institute Pasteur (IP)	Undetected			
IP 689	Institute Pasteur (IP)	Undetected			
IP 690	Institute Pasteur (IP)	Undetected			
IP 691	Institute Pasteur (IP)	Undetected			
IP 692	Institute Pasteur (IP)	Undetected			
IP 693	Institute Pasteur (IP)	Undetected			
IP 694	Institute Pasteur (IP)	Undetected			
IP 695	Institute Pasteur (IP)	Undetected			

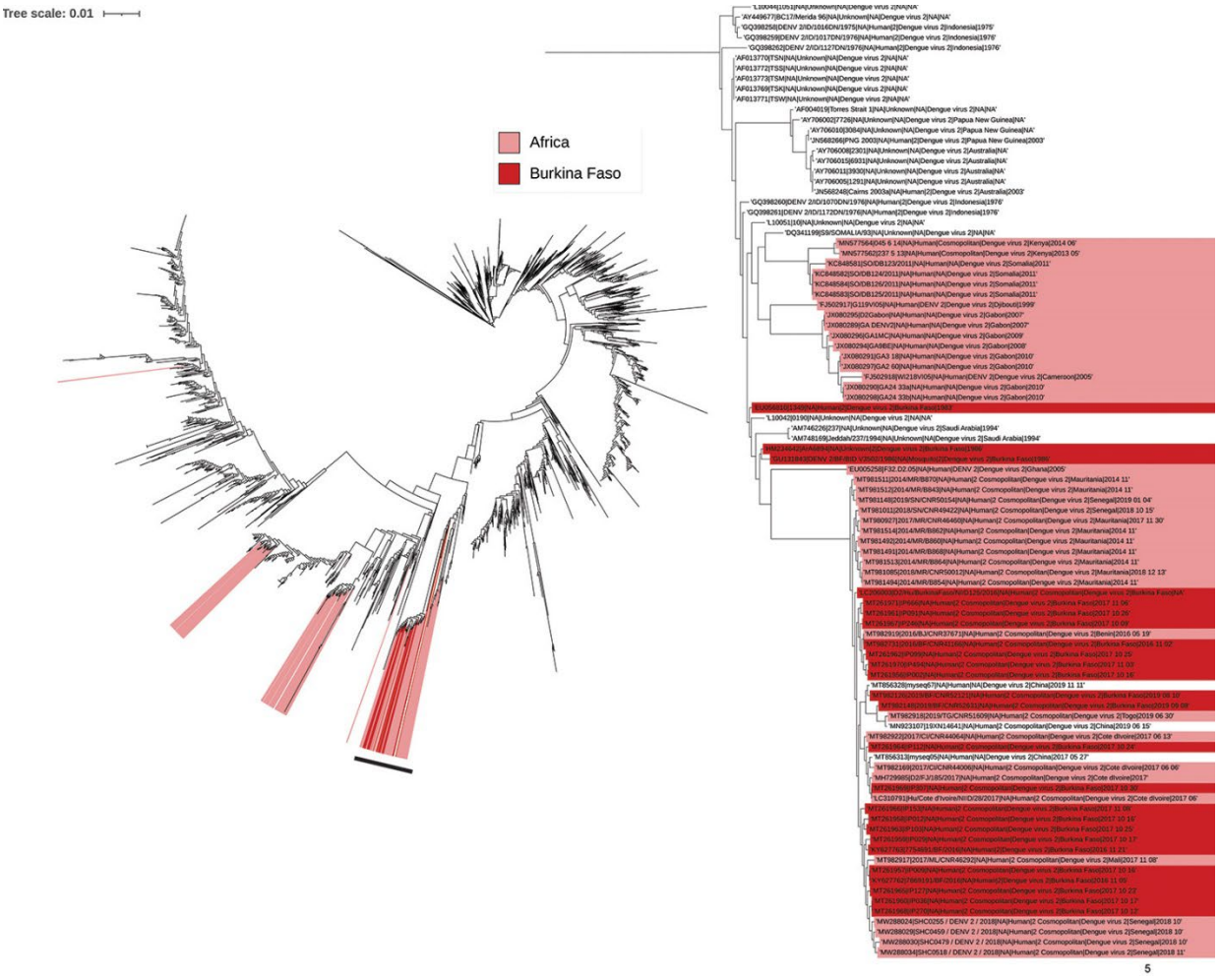
NMIMR Lab ID	Name of site	Trioplex rRT-PCR ct	Serology		
			Ag NS1	IgM	IgG
IP 696	Institute Pasteur (IP)	Undetected			
IP 697	Institute Pasteur (IP)	Undetected			
IP 698	Institute Pasteur (IP)	Undetected			
IP 699	Institute Pasteur (IP)	Undetected			
IP 700	Institute Pasteur (IP)	Undetected			
IP 701	Institute Pasteur (IP)	Undetected			
IP 702	Institute Pasteur (IP)	Undetected			
IP 703	Institute Pasteur (IP)	Undetected			



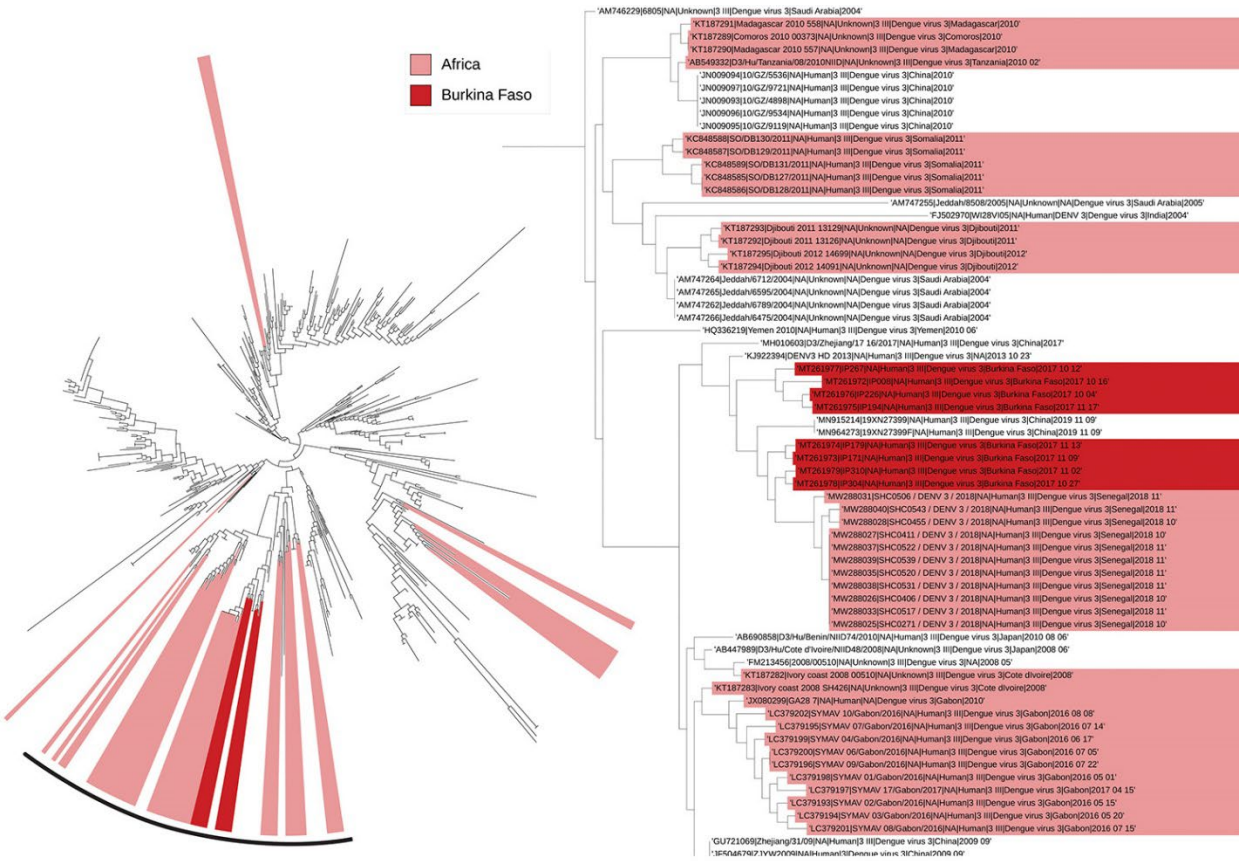
Appendix Figure 1. Sample testing workflow.



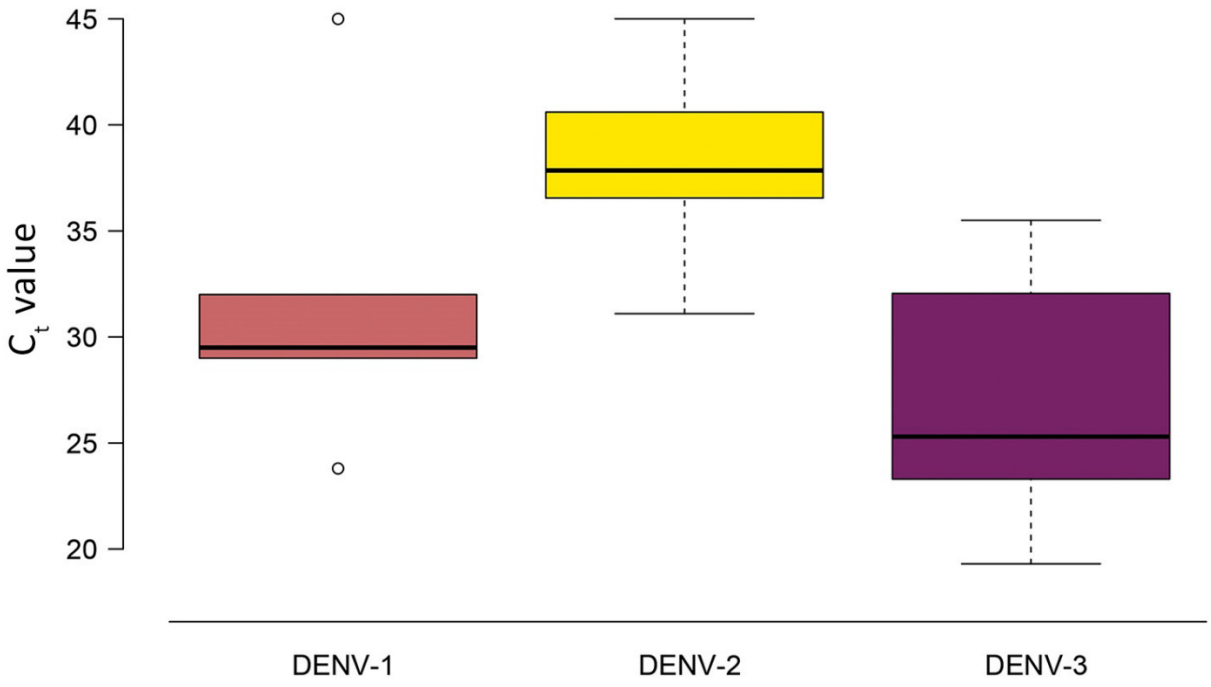
Appendix Figure 2. Dengue virus serotype 1 phylogenetic tree inferred from all available DENV-1 sequences with E-gene coverage. Presented circular tree is a subtree including only genotype V. The tree on the right is a further subtree as indicated by the black bar. Genomes highlighted in pink originated from countries in Africa and those in red from Burkina Faso. When all known sequences are included in the tree, conclusions from the whole genome analysis are still supported.



Appendix Figure 3. Dengue virus serotype 2 phylogenetic tree inferred from all available DENV-2 sequences with E-gene coverage. Presented circular tree is a subtree including only genotype Cosmopolitan. The tree on the right is a further subtree as indicated by the black bar. Genomes highlighted in pink originated from countries in Africa and those in red from Burkina Faso. When all know sequences are included in the tree, conclusions from the whole genome analysis are still supported.



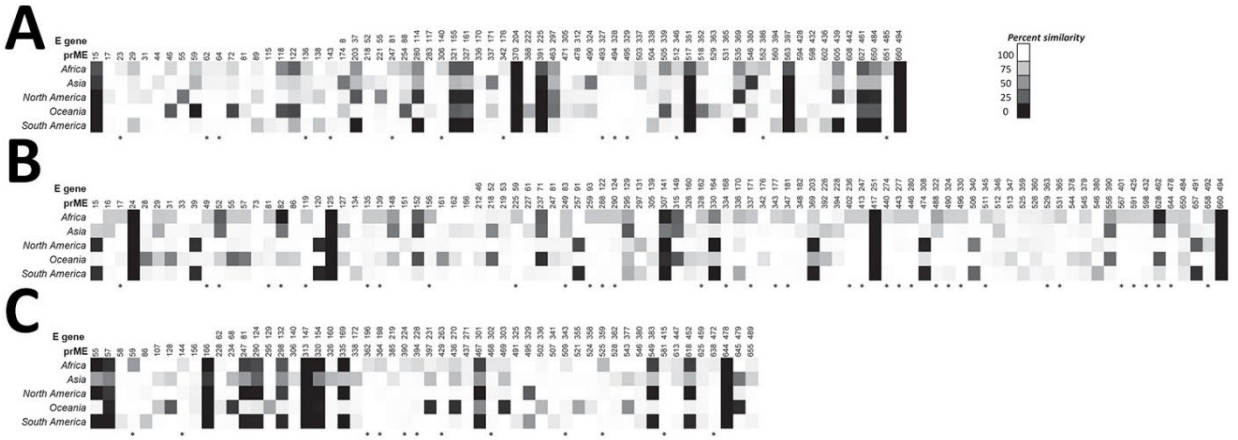
Appendix Figure 4. Dengue virus serotype 3 phylogenetic tree inferred from all available DENV-3 sequences with E-gene coverage. Presented circular tree is a subtree including only genotype III. The tree on the right is a further subtree as indicated by the black bar. Genomes highlighted in pink originated from countries in Africa and those in red from Burkina Faso. When all known sequences are included in the tree, conclusions from the whole genome analysis are still supported.



Appendix Figure 5. Box plots of Trioplex rRT-PCR Ct values for the 29 genomes with over 85% coverage from the 2017 Burkina Faso dengue virus outbreak. Undetected samples were designated a Ct of 45. The Trioplex rRT-PCR exhibits a higher median Ct against DENV-2, than DENV-1 or −3, indicating lower sensitivity toward DENV-2 sequences. Of note for DENV1, only 5 values were available therefore the upper and lower values are represented by points.

		DENV1	DENV2	DENV3	Gene target
Johnson et al.	*Forward*	0	1	1	**DENV-1: NS5 gene**
	Reverse	1	2	0	**DENV-2: E gene**
	Probe	0	3	0	**DENV-3: prM gene**
CDC-DENV-1-4	*Forward*	0	0	0	**DENV-1: NS5 gene**
	Reverse	2	1	0	**DENV-2: E gene**
	Probe	0	2	0	**DENV-3: prM gene**
Trioplex	*Forward*	0	1	0	
	Reverse1	0	1	0	**5' UTR/C gene**
	Probe	0	0	0	

Appendix Figure 6. Heatmap indicating the number of mismatches between dengue virus molecular diagnostics and circulating dengue virus genomes. Burkina Faso dengue virus outbreak genomes from 2017 were compared to the Trioplex, CDC-DENV-1–4, and Johnson et al. primer-probe sequences within the primer-probe binding sites. All three diagnostics have mismatches against the 2017 Burkina Faso dengue virus outbreak genomes, especially against DENV-2 genomes.



Appendix Figure 7. Conserved site analysis between the Dengvaxia® (CYD-TDV) vaccine and all A) DENV-1, B) DENV-2, and C) DENV-3 genomes trimmed to the prM and E gene region, and split by continent. Only amino acid positions with more than 5% divergence in at least one continent are shown for clarity. The darker the color, the greater the proportion of DENV genomes within that continent that are divergent from the vaccine amino acid at that position. Amino acid positions where African genomes are the most divergent are marked with an asterisk.

Appendix Figure 8. Conserved site analysis between the TetraVax-DV-TV003 (TV003) vaccine and all A) DENV-1, B) DENV-2, and C) DENV-3 genomes trimmed to the prM and E gene region, and split by continent. Only amino acid positions with more than 5% divergence in at least one continent are shown for clarity. The darker the color, the greater the proportion of DENV genomes within that continent that are divergent from the vaccine amino acid at that position. Amino acid positions where African genomes are the most divergent are marked with an asterisk.